SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME PEPTIDE COUPLED QUINAZOLIN-4(3H)-ONES

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University

Chennai- 600032

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

Submitted by

V. BHARATHI

Reg.No. 26106031

Under the Guidance of

Mr. A. THIRUGNANA SAMBANTHAN, M.Pharm., (Ph.D.)

Assistant Professor

Department of Pharmaceutical Chemistry

ADHIPARASAKTHI COLLEGE OF PHARMACY

(Accredited By "NAAC" with CGPA of 2.74 on a Four Point Scale at "B" Grade)

MELMARUVATHUR- 603319

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CERTIFICATE

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Place: Melmaruvathur

Date:

Mr. A.THIRUGNANA SAMBANTHAN, M.Pharm., (Ph.D.)
Assistant Professor,
Department of Pharmaceutical Chemistry,
Adhiparasakthi College of Pharmacy,
Melmaruvathur-603319.

CERTIFICATE

This is to certify that the dissertation entitled **"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME PEPTIDE COUPLED QUINAZOLIN-4(3H)-ONES"** is the bonafide research work carried out by **V. BHARATHI** in the Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur, which is affiliated to The Tamilnadu Dr. M.G.R Medical University under the guidance of **Mr. A. THIRUGNANA SAMBANTHAN, M.Pharm., (Ph.D.)** Assistant Professor, Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

Place: Melmaruvathur
Date:

Prof. (Dr.). T. VETRICHELVAN, M.Pharm., Ph.D.,
Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur-603319.

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Department of Pharmaceutical Chemistry and other faculty members of Adhiparasakthi college of Pharmacy, Melmaruvathur, for their valuable help and guidance during the course of my research work.

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I am indeed very much thankful to the librarian **Mr. M. Suresh, M.L.I.S.,** for providing all reference books for the completion of this project.

My Sincere thanks and appreciation to my beloved parents, sisters, friends and relatives for their support, sacrifice and pain they have taken in bringing me up to this position.

V. BHARATHI

DEDICATED TO GOD AND MY BELOVED PARENTS

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ABBREVIATIONS

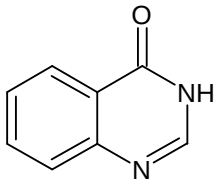
Gly	-	Glycine
Phe	-	Phenyl alanine
Leu	-	Leucine
Val	-	Valine
Ala	-	Alanine
Boc	-	Tert-butloxycarbonic anhydride
IPA	-	Iso propyl alcohol
DCM	-	Dichloro methane
TEA	-	Tri ethyl amine
EDC	-	1-ethyl-3-(3dimethylaminopropyl) Carbodiimide hydrochloride
RT	-	Room temperature
M.P	-	Melting point
R_f	-	Retardation factor
m.mol	-	Millimoles
g	-	Grams
ml	-	Milli litre
ppm	-	Parts per million
IR	-	Infra Red spectroscopy
^{1}H NMR	-	Proton Nuclear Magnetic Resonance spectroscopy
^{0}C	-	Degree Celsius
hrs	-	Hour
TLC	-	Thin layer chromatography

INTRODUCTION

I. INTRODUCTION

QUINAZOLONE

Quinazolinone ring is an aromatic benzopyrimidine system, earlier known as benzo-1, 3-diazine was prepared in the laboratory by Gabriel in 1903. It is yellow and crystalline in nature.



The recent literature contains much information concerning the synthesis and pharmacological activity of the quinazolinone. The quinazolinone skeleton is a building block for the preparation of natural purine base, alkaloids, many biologically active compounds and intermediates in organic synthesis.

The quinazolinone moiety is an important pharmacophore showing many types of pharmacological activites such as anticancer *(V. Murugan et al., 2003),* antibacterial *(K. R. Desai et al., 2006),* anti HIV *(V. Alagarswamy et al., 2003),* antiinflammatory *(E. M. Jessy et al., 2007),* antifungal *(M. K. Shivananda et al., 2011),* analgesic *(K. Hemalatha et al., 2011),* antihyperglycemic *(Vishnu Ji Ram., et al., 2003),* anticonvulsant *(Desai K. R., et al 2003)* etc.

Compounds containing a fused quinazolinone ring belong to a broad class of compounds which have received a considerable attention over the past years due to their wide range of biological activities. Some of aminoquinazolinone derivatives were found to be cardiac stimulants, they were also found as inhibitors of the tyrosine kinase or dihydrofolate reductase enzymes so they work as potent anticancer agents.

They are also used for hypertension, malaria and to fight infections involving AIDS. Besides biological activity, quinazolinones have O and N donor atoms, so they can act as good chelating agents. Many efforts have been focused on the modification of structure of quinazolinone for development of clinically suitable compounds. Both naturally occurring and synthetic quinazolines and quinazolinones have attracted widespread attention due to their diverse range of pharmacological activities.

These structures are defined by the location of the oxygen and the hydrogen on the nitrogen. Of the many derivatives of quinazoline system known so far, keto-quinazolines also called as quinazolinones, are the most important compounds. Depending upon the position of the keto or oxo group, these compounds may be classified into two types: 2-(1H) quinazolinones (or) 1, 2-dihydro-2-oxo quinazolines and 4(3H)-quinazolines or 3, 4-dihydro-oxoquinazoline. These systems exhibit lactam-lactim tautomerism and undergo hydroxl group replacement reactions. 2-Cyano-4(3H)-quinazolinone was the first quinazolinone derivative to be synthesized.

Quinazolinone chemistry is considered to be an established area, newer and more complex variants of the quinazolinone structures are still being discovered. The first reported synthesis of a quinazolinone occurred in 1869, which was prepared from anthranilic acid and cyanide in ethanol creating 2-ethoxy-4(3H)-quinazolinone. These findings were confirmed by the preparation of the derivatives of 2-amino-4(3H)-quinazolinone and 2, 4 (1H, 3H)-quinazoline dione by reactions with ammonia and water respectively. Investigations of quinazolinones show there is a strong lactam-lactim tautomeric interaction.

The significance of this tautomeric interaction can also be seen when a 4(3H)-quinazolinone containing a methyl in the 3-position is subjected to chlorination with $POCl_3$, the methyl group is lost and chlorination proceeds; and when the methyl group is present in the 2-position, the tautomeric effect is extended generating an exomethylene carbon, this compound can be condensed with aldehydes producing 2-styryl-4(3H)-quinazolinones. The significance of these extended tautomeric effects is that they enhance reactivity of the substituted 4(3H)-quinazolinones and are considered to be a privileged structure for drug development. A structure activity relationship study of quinazolinone ring system reveals that position 2, 6 and 8 are very much important for structure activity studies and position 3 should be attached to different heterocyclic rings for better chemotherapeutic activity.

Quinazolinones are high melting crystalline solids, insoluble in water and in most organic solvents but soluble in aqueous alkali. They are generally insoluble in dilute acids but are soluble in concentrated acids. Simple 4(3H)-quinazolinones, although insoluble in dilute acids, are soluble in 6N hydrochloric acid. 4(3H)-quinazolinones form stable monohydrochlorides, chloroplatinate, chloroaurates and picrates and their metal salts of silver, mercury, zinc, copper, sodium and potassium.

The ring system in quinazolinone is exceedingly stable in oxidation, reduction and hydrolysis reactions. There is no report of degradation of quinazolinone by simple chemical oxidation.

Most of the methods employed for the synthesis of 4(3H)-quinazolinones make use of anthranilic acid or one of their functional derivatives as the starting materials. Based on this factor, the general methods of synthesis are:

Condensation of anthranilic acid with acid amides:

When anthranilic acid is heated in a open container with excess of formamide at 120°C, water is expelled and a nearly quantitative (90%) conversion to 4(3H)-quinazolinones is achieved.

Condensation of acetanilides with urethanes:

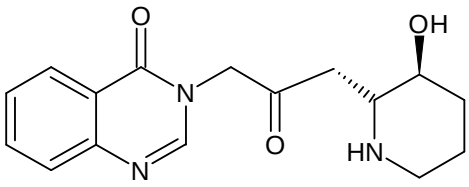
A number of attempts have been made to condense a urethane derivative with aniline to give 4-(3H)-quinazolinone directly. When Urethane and acetanilide heated for 3 hours with phosphorus pentoxide in toluene gives 2-methyl-4(3H)-quinazolinone. Condensation of N-acylanthranilic acids with primary amines:

4(3H)-Quinazolinones may also be synthesized directly from the corresponding N-acylanthranilic acid by heating with ammonia or substituted amines.

Drugs containing quinazolone nucleus

Febrifugine

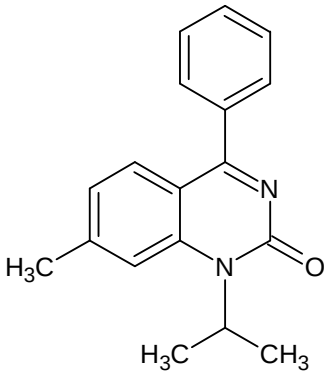
It has antimalarial properties and the halogenated derivative halofuginone is used in veterinary medicine as a coccidiostat.



Febrifugine

Praquazone

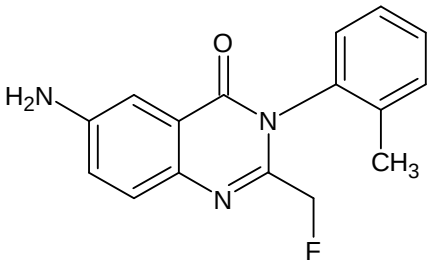
It is used as non steroidal antiinflammatory drug



Praquazone

Afloqualone

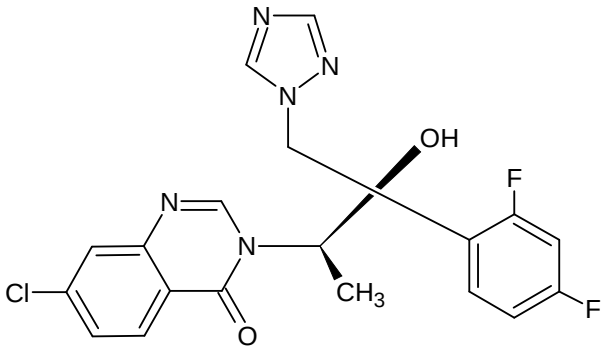
Its brand name is Arofuto. It has sedative and muscle relaxant effects.



Afloqualone

Albaconazole

It is a triazole antifungal and was potent broad-spectrum antibacterial activity.



Albaconazole

Apart from this the other compounds containing quinazolone nucleus are prazosin, anagrelide, praquazone, nolatrexed etc.

PEPTIDES

A peptide consist of two or more amino acids linked by a peptide bond between carboxylic group of one amino acid and the amino group of another with removal of one mole of water. These are short polymers formed from the linking in a defined order of alpha amino acids. The link between one amino acid residue and the next is called an amide bond or peptide bond. Depending upon the number of amino acids residue per molecule, they are classified as dipeptides, tripeptides and tetrapeptides. *(Rio Y. V et al., 1986)*

The monomers present as repeated units in peptides and proteins are α-amino acids. The α-carbon ia asymmetric, bonded to four different substitutent groups and is thus a chiral center. Today 20 amino acids are known as genetically encoded as building blocks of peptides and proteins. Almost all of them present in peptides have L-configuration. D-amino acids have been found only in small peptides of bacterial cell walls, peptide antibiotic and peptides in South American frog skin.

Synthetic peptides are used as drugs and diagnostic agents. Peptide drugs are either naturally-occuring peptides or altered natural peptides. There are many naturally occuring peptides that are biologically active. In addition, the amino acids in an active peptide can be altered to make analogues of the original peptide. If the analogue is more biochemically active than the original peptide it is known as an agonist and if it has the reverse effect is known as an antagonist.

Biologically active peptides range in size from molecules containing only two or three amino acids to large molecules containing several tens of amino acids. Among them, neuropeptides, hypothalamic hormones, proteohormones of the pituitary, thyroid hormones, gastrointestinal peptides, muramyl peptides, peptides of immunological significance, peptide vaccines, plasma kinins, atrial nutriuretic peptides, peptide antibiotics, peptide toxins, peptide insecticides and herbicides are apparent.

Actinomycin belongs to a class of chromopeptides and is characterized by its cytostatic growth inhibition in tumour and for antibacterial action. Cyclosporin A, the potent immunosuppressant is currently used for preventing rejection of transplanted human organs. Atrial natriuretic peptides have diuretic properties. Several muramyl peptides have the possibility of being used as adjuvents in combination with vaccines or with antibiotics. Albomycins are nucleopeptides which possess iron complexing properties. ***(****E Schroder et al., 1965****)***

Carnosine and the related dipeptides anserine have appeared to exist in many tissues of mammalian, bird, and fish origin. A lot of functions have been anticipated to such dipeptides, like antioxidation and maintenance of cellular pH. Since dipeptides and

their derivatives reflect such possible functions, they are used in many ways - in sport nutrition, for example, which is based on the fact that the muscle of a fast swimming fish contains the dipeptides in higher concentrations. Other compounds, like Zinc carnosine, can be used as an antiulcer drug, while N-acetyl carnosine is used as an agent for cataracts. Kyotorphin was isolated from bovine brain to feature analgesic effects; a synthetic dipeptides Lys-Glu was found to show antitumor activity, while Leu-Ile appeared to have a neuroprotective effect, and Tyr-Gly is known for enhancing proliferation of peripheral blood lymphocytes. *(WWW. Wikipedia. com)*

Analgesics commonly known as pain killers, the term used for any member of the group of drugs that are used to relieve pain and to achieve analgesia or painless state. Analgesic drugs show their activity by acting on peripherial or central nervous system. The relief of pain induced by analgesics occurs by blocking of pain signals reaching the brain.

Analgesics are classified into opioid or narcotic analgesics and Non opioid analgesics or non-steriodal anti inflammatory drugs. The term opioid refers to drugs derived from opium poppy, whereas 'opioid analgesic' applies to any substances endogenous peptides or drugs. Natural opium alkaloids are morphine, codine, thebaine, semisynthetic opioids are heroin, hydromorphone, oxymorphone and synthetic opioids are pethidine, methadone, propoxyphene, levorphanol and tramadol.

Opioid drugs produce actions by interacting with various opioid receptors- mu (μ), delta (δ) and kappa (κ). They are located at spinal, supraspinal and peripherial nerves.

Non opioid analgesics are synthetic in nature and are less potent compared to narcotic analgesic. They are mainly used for mild to moderate pains.

Endogenous opioid peptides are naturally occurring substances present in brain and other body tissues eg: endorphins, enkephalins and dynorphins. These peptides appear to be involved in placebo and acupuncture-induced analgesia. *(Tara V Shanbhag et al., 2008)*

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoas. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

There are mainly two classes of antimicrobial drugs:

Those obtained from natural sources: Beta-lactam antibiotics (such as penicillins, cephalosporins) and protein synthesis inhibitors (such as aminoglycosides, macrolides, tetracyclines, chloramphenicol, polypeptides).

Synthetic agents: Sulphonamides, cotrimoxazole, quinolones etc. They act as antiviral, antifungal, anticancer, antimalarials, antituberculour, antileprotics, and antiprotozoal agents.

Antimicrobial peptides (also called host defense peptides) are an evolutionarily conserved component of the innate immune response and are found among all classes of life. Fundamental differences exist between prokaryotic and eukaryotic cells that may represent targets for antimicrobial peptides. These peptides are potent, broad spectrum

antibiotics which demonstrate potential as novel therapeutic agents. Antimicrobial peptides have been demonstrated to gram negative and gram positive bacteria (including strains that are resistant to conventional antibiotics), mycobacteria (including *Mycobacterium tuberculosis*), enveloped viruses, fungi and even transformed or cancerous cells.

Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. Antimicrobial peptides are generally between 12 and 50 amino acids.

In addition to killing bacteria directly, they have a number of immunomodulatory functions that may be involved in the clearance of infection, including the ability to alter host gene expression, act as chemokines and/or induce chemokine production, inhibiting lipopolysaccharide induced pro-inflammatory cytokine production, promoting wound healing, and modulating the responses of dendritic cells and cells of the adaptive immune response. Animal models indicate that host defence peptides are crucial for both prevention and clearance of infection. A number of naturally occurring peptides and their derivatives have been developed as novel anti-infective therapies for conditions as diverse as oral mucositis, lung infections associated with cystic fibrosis (CF), cancer, and topical skin infections. *(WWW. Wikipedia. Com)*

Based on their reports, we have planned to synthesize the quinazolone by incorporating dipeptide and derive a set of peptido quinazolones and to screen for analgesic and antimicrobial activities.

OBJECTIVES OF THE WORK

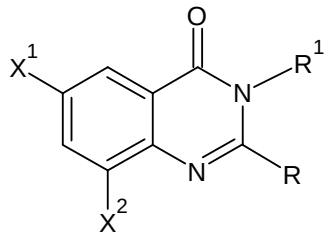
II. OBJECTIVES OF THE WORK

- To synthesize 2-styryl-3-peptido quinazolone derivatives by condensation of different aromatic aldehydes with 2-methyl-3-peptido quinazolone which is prepared from 2-methyl-4H-3,1-benzoxazin-4-one and dipeptide.
- To characterize the structures of synthesized compounds by IR, ^{1}H NMR and MASS spectroscopic techniques.
- To screen the analgesic activity of synthesized compounds by Eddy's hot plate method.
- To screen the antibacterial and antifungal activity of synthesized compounds by disc diffusion method.
- To interpret and conclude the results obtained from the study.

LITERATURE REVIEW

III. LITERATURE REVIEW

Hemalatha K. *et al.*, (2011) synthesized some novel 2,3 disubstituted quinazolinone derivatives and screened for their analgesic and antiinflammatory activities.

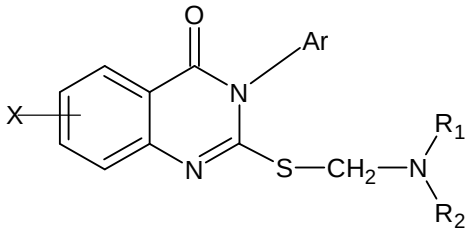


X^1, X^2= H, Br,

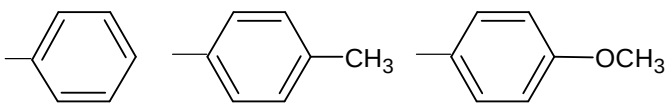
R= CH_3, C_6H_5,

R^1= -$NHCOC_6H_5$, -N $(COC_6H_5)_2$.

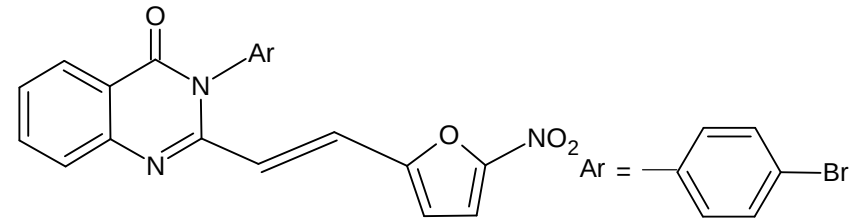
Kumaraswamy D. *et al.*, (2011) synthesized some new 2-S-substituted amino methyl thio-3-aryl-4-(3H) quinazolinones and evaluated for their antibacterial, antifungal, and analgesic activities.



X=H, Br, -N R_1 R_2= -N-$(CH_3)_2$, -N-$(C_6H_5)_2$.

Ar = 

Shivananda M. K. *et al.*, (2011) synthesized a series of quinazolinones carrying nitrofuran moiety and studied their antifungal properties.



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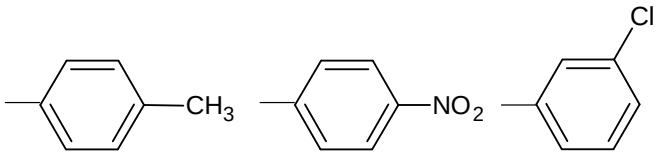

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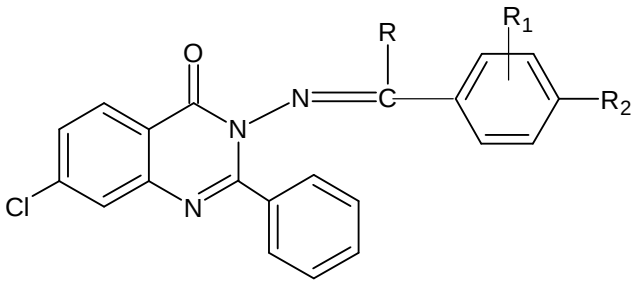
Vijai Anand P. R. *et al.*, (2011) synthesized a series of novel (E)-7-chloro-3-substituted-2-phenyl quinazolin-4-(3H)-one derivative was found to have significant effect against the antibacterial activity.

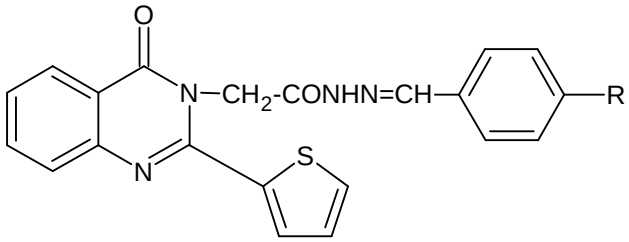


R= H, CH_3.

R_1= H, 4-OH, 2-OH, 3-OCH_3, 4-N $(CH_3)_2$,

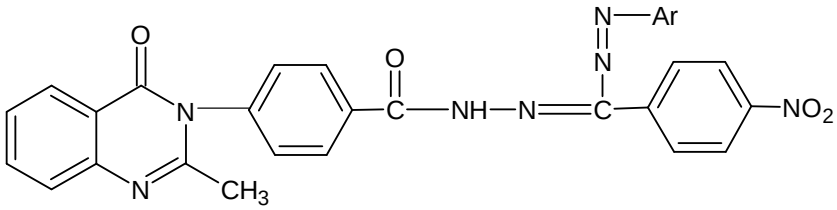
R_2 = H, 4-OH.

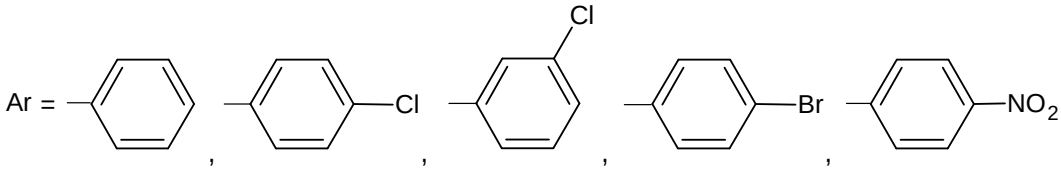
Praveen Kumar P. *et al.*, (2011) synthesized some new schiff bases for antimicrobial activity containing 2-thienyl-3-arylidine substituted -4-(3H)-quinazolinone derivatives.



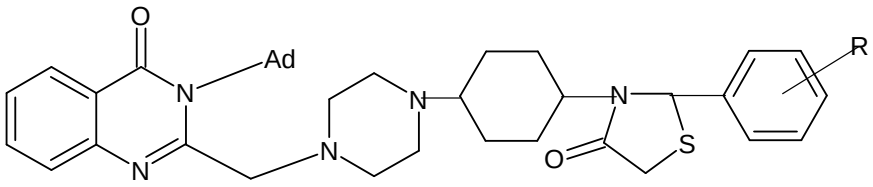
R= OCH_3, CH_3, Cl, CH_3, OH, NO_2.

Narendra Babu A. *et al.*, (2011) synthesized some novel 1-substituted phenyl-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazans exhibiting prominent antimicrobial and analgesic activities.





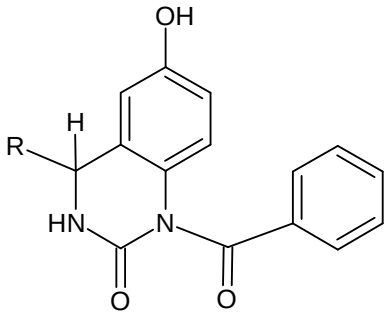
Hemant Panwar *et al.*, (2011) synthesized some novel 4-(3H)-quinazolinone derivatives incorporating bioactive moiety such as adamantine, piperazine, imidazolidine, and thiazolidinone and evaluated them for antibacterial, antifungal, antiinflammatory analgesic activities.

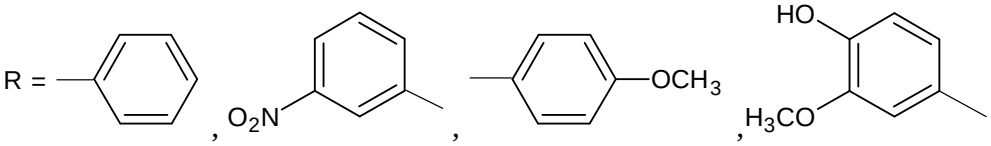


R = C_6H_5, 2-Cl C_6H_4, 4- Cl C_6H_4, 2-OCH_3 C_6H_4, 3-OCH_3 C_6H_4, 4-OCH_3 C_6H_4.

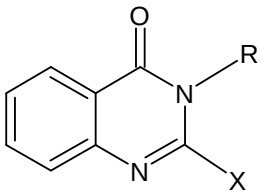
Ad = Adamantine

Vijey Anandhi M. *et al.*, (2011) synthesized 3,4-dihydro quinazolin-2-(H)-one derivatives and evaluated for analgesic and antibacterial activities.

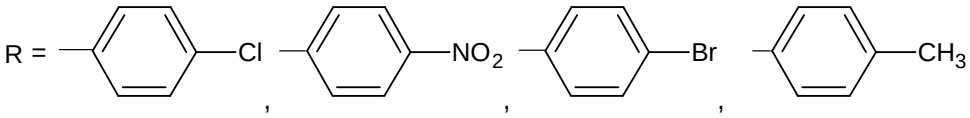




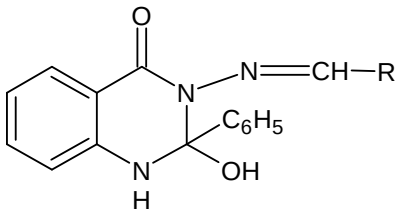
Manasa K. *et al.*, (2011) synthesized 2,3 disubstituted quinazoline derivatives and screened for antioxidant and anticancer activities.

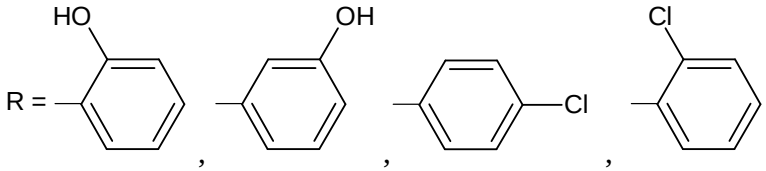


X= C_6H_5

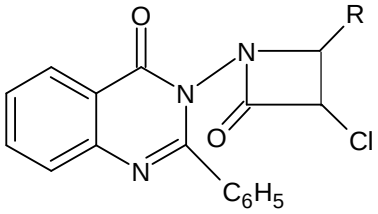


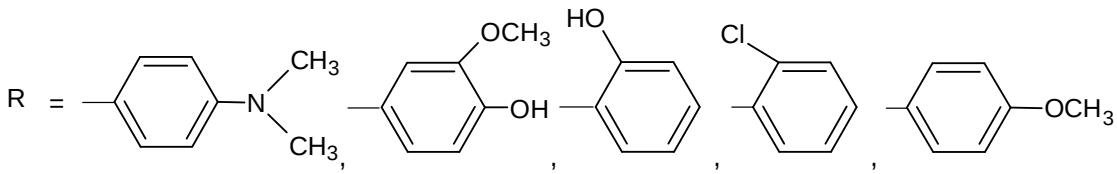
Pankaj S. Salunkhe *et al.*, (2010) synthesized 2-phenyl-2, 3-dihyroquinazoline-4(1H)-one derivatives showing good to moderate analgesic and antiinflammatory activities.



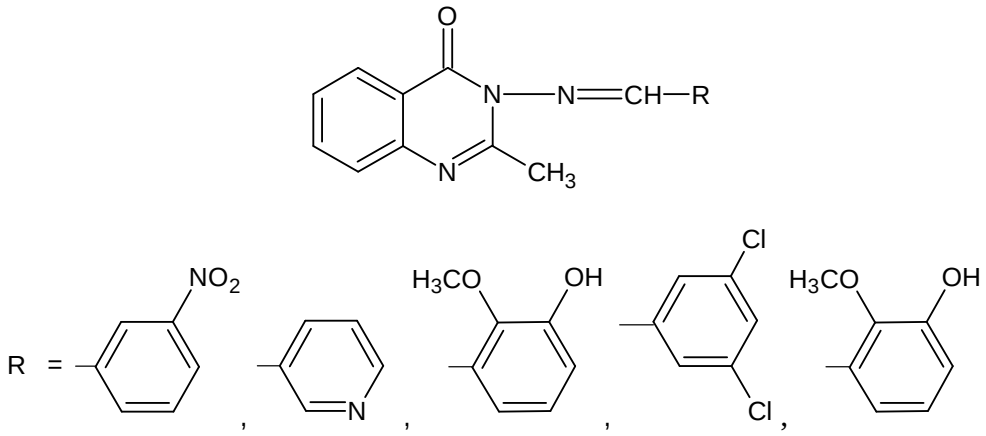


Beena K. P. *et al.*, (2010) synthesized quinazolinone derivatives and showing potent antibacterial activity.

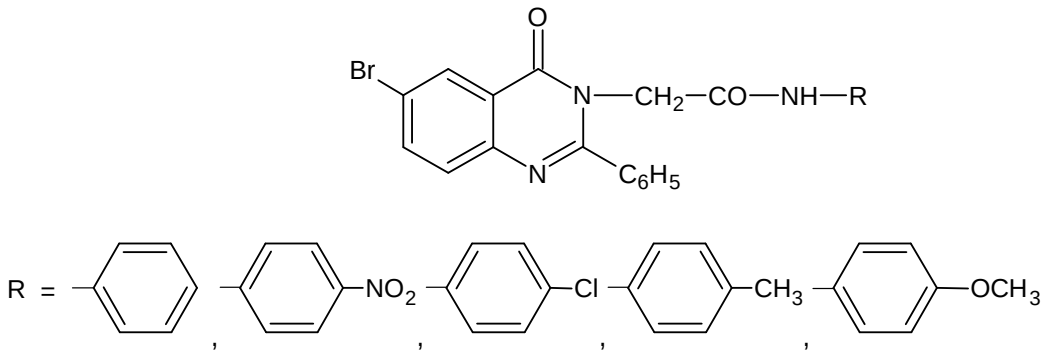




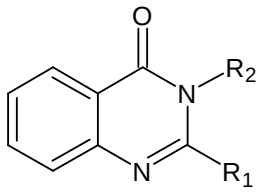
Hosakere D. Revanasiddappa *et al.*, (2010) synthesized a new series of schiff bases of 3-amino-2-methyl-3-substituted-4(3H) quinazolinone for antimicrobial and antihelmintic activities.



Rajveer CH. *et al.*, (2010) synthesized 2-[6-bromo-2-phenyl-4-oxoquinazolin-3(4H)-yl]-N-substituted acetamide derivatives and performed antibacterial, anti-inflammatory and analgesic activities.

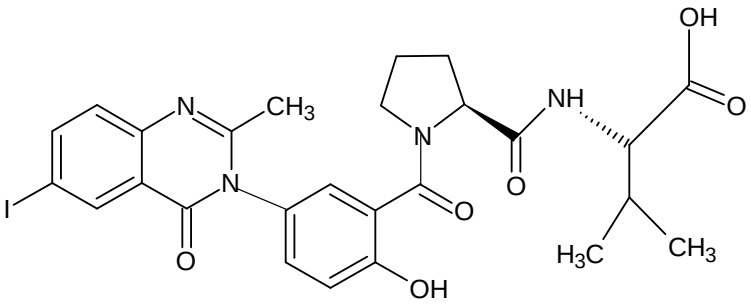


Akhil A. Nagar *et al.*, (2010) synthesized 2,3 disubstituted quinazolin-4-(3H)-ones by microwave assisted one pot synthesis and reported for the antibacterial and antifungal activities.

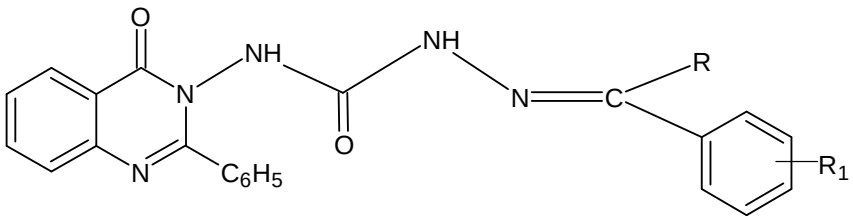


R_1= CH_3, R_2 = CH_3, C_2H_5, C_6H_5, C_6H_5OH.

Kaur P. *et al.*, (2010) applied new approach of quinazolinone peptides synthesis showing potent antitumour, antimicrobial, analgesic, antiinflammatory and antihelmintic activities.



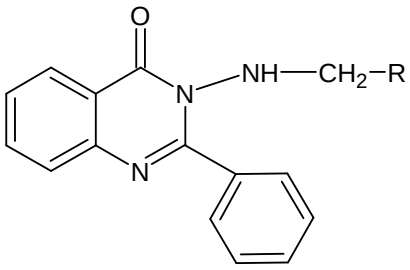
Ponnilavarasan Ilangovan *et al.*, (2010) synthesized a series of substituted quinazolinone semicarbazone at third position and studied for their anticonvulsant activity.

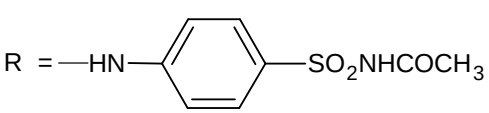


R = H, CH_3

R_1 = H, 4-Cl, 4-N $(CH_3)_3$, 3-OCH_3, 4-OH, 3-NO_2, 2-OCH_3, 2-OH.

Sakthi Saravanan *et al.*, (2010) synthesized a series of novel 2-phenyl-3-substituted quinazolin-4-(3H) one derivatives and screened for antiviral activity against panel of human pathogenic viruses.





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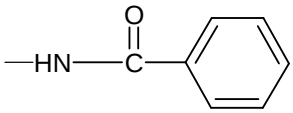

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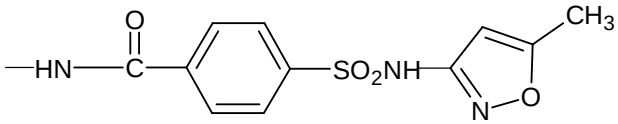
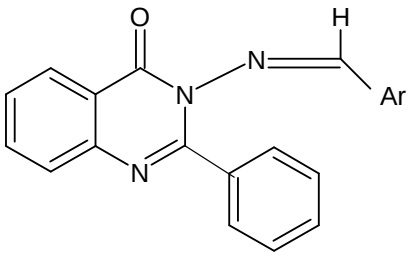
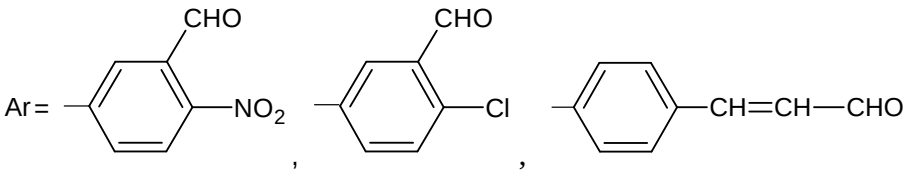
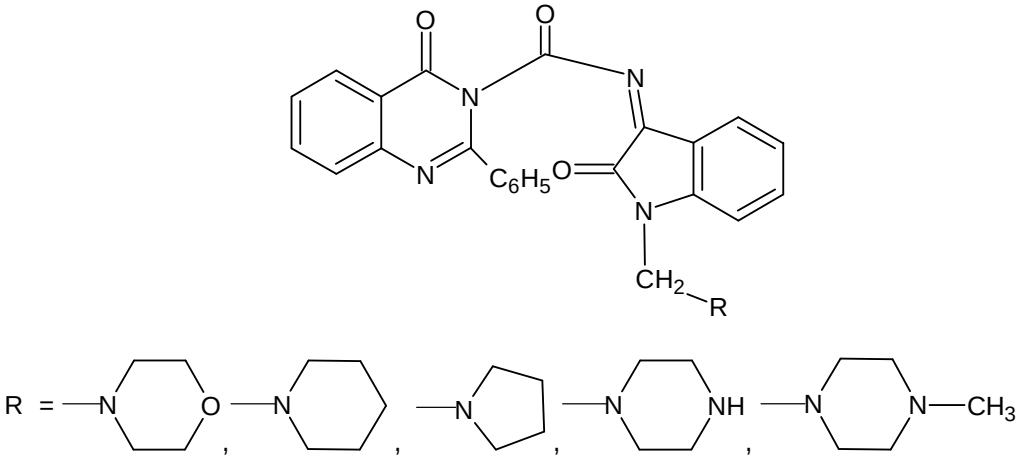


Marriappan G. *et al.*, (2010) synthesized some 2-phenyl-3-amino quinazolin-4-(3H)-one followed by converted to Schiff bases and found to possess antiinflammatory, antibacterial and antifungal activities.

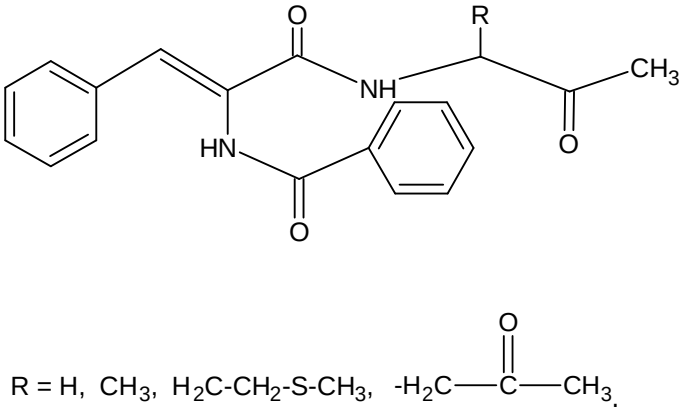




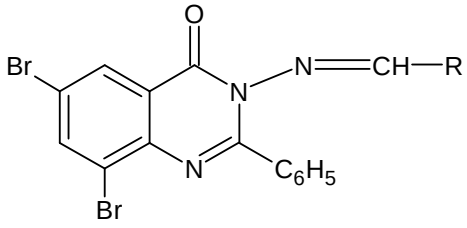
Ilango K. *et al.*, (2010) synthesized a newer quinazolin-4-(3H)-one clubbed with isatin derivatives as potent antimicrobial agents.

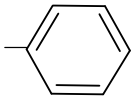
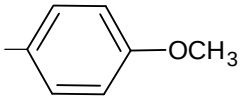
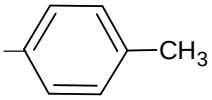


Haseena Banu B. *et al.*, (2010) synthesized some N-protected dehydrophenylalanine dipeptides and evaluated for analgesic activity.

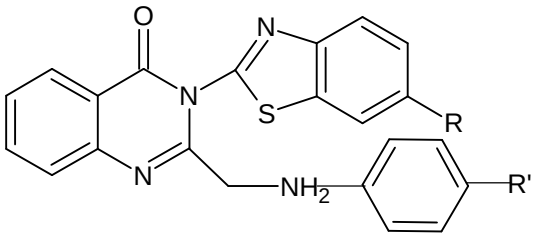


Panneerselvam P. *et al.*, (2009) synthesized some schiff bases of 3-amino-6,8-dibromo-2-phenyl quinazolin-4-(3H)-ones and reported for antibacterial and antifungal activities.



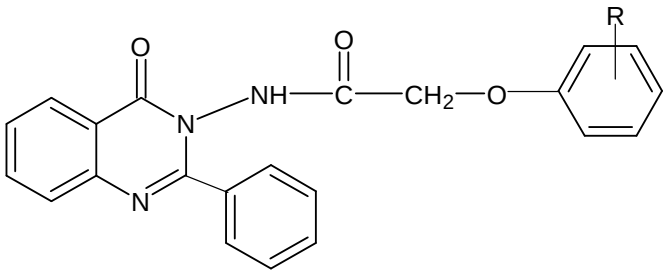
R =  ,  , 

Salahuddin M. D. *et al.*, (2009) synthesized some novel 3-(6-substituted-1,3-benzothiazole-2-yl)-2-[{(4-substitutedphenyl)amino}methyl]quinazolin-4(3H)-ones and investigated for their antiinflammatory activity.



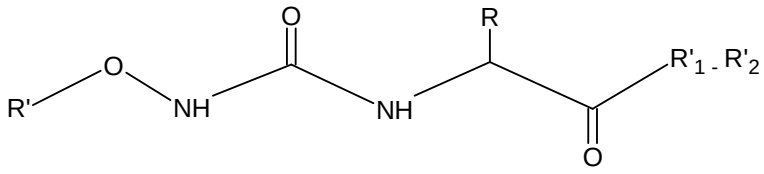
R= CH_3, OCH_3, Cl, F. R'= H, CH_3, OCH_3, Cl.

Deepti Kohli *et al.*, (2009) synthesized new series of 2-phenyl-3-substituted quinazolinone derivatives and showing potent antibacterial activity.

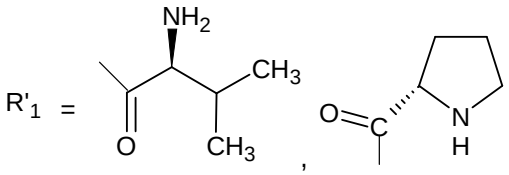


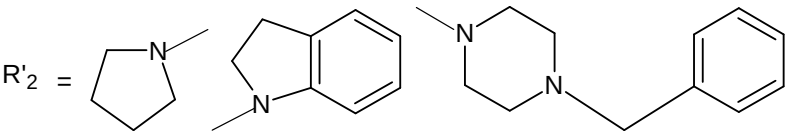
R= H, 4-NO_2, 4-Cl, 2,6- Cl, 2-$COOCH_3$.

Ming Kuan Hu *et al.*, (2009) synthesized N-(hydroxyl) urea-based dipeptide as potential antibacterial agents.

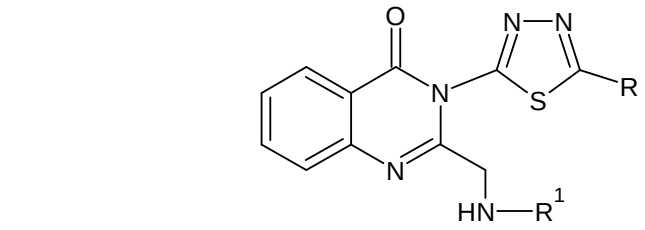


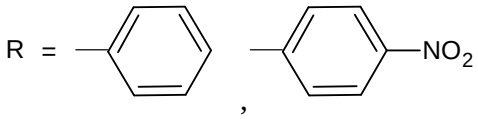
R= C_6H_5, H R'= $CH_2CH(CH_3)_2$, $CH_2CH_2CH_2CH_3$, $CH_2CH_2SCH_3$

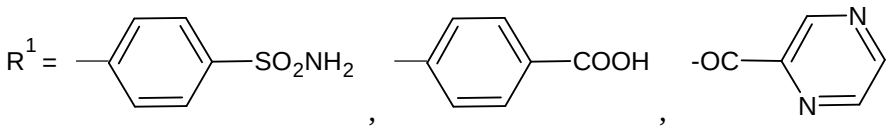




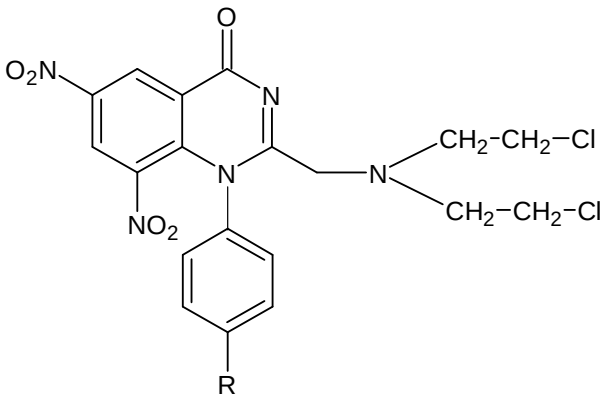
Jayshari S Pattan *et al.*, (2009) synthesized some new N^1-3-(5-substituted-1,3,4-thiadiazol-2-yl)-(2-aminomethyl)-quinazolin4(3H)-one derivatives and reported for antimicrobial activity.





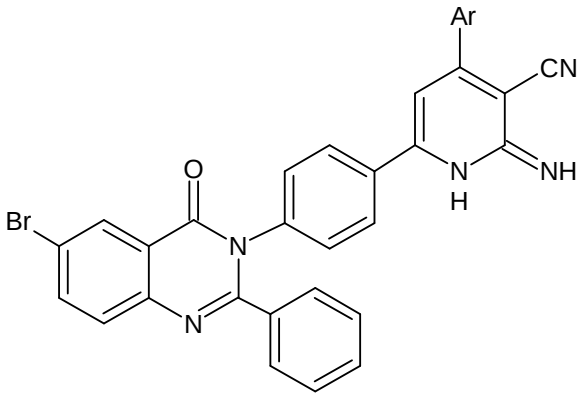


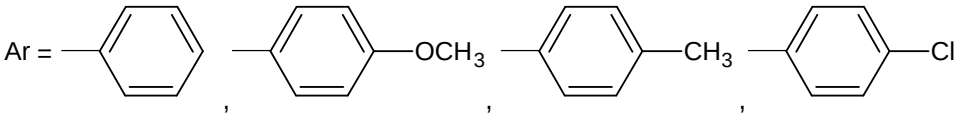
Yuvaraj Govindaraj *et al.*, (2009) synthesized 2-{[Bis-(2-chloroethyl) amino]methyl}-6,8 dinitro-1-(4-substituted ethyl)-1H-quinazolin-4-one derivatives and evaluated for in-vivo anticancer activity.



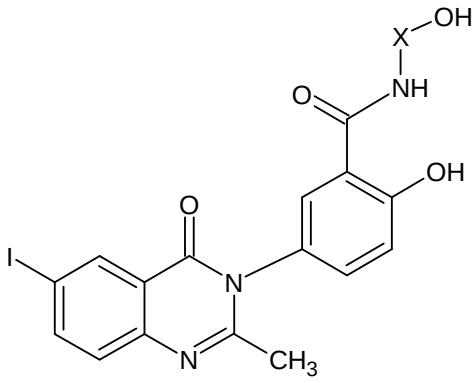
R= H, CH3, Cl, OCH_3, OC_2H_5, NO_2.

Mosaad S. Mohamed *et al.*, (2009) synthesized new series of novel 3-(P-substituted phenyl)-6-bromo-4(3H)-quinazolinone derivatives showing promising analgesic and anti-inflammatory activities.



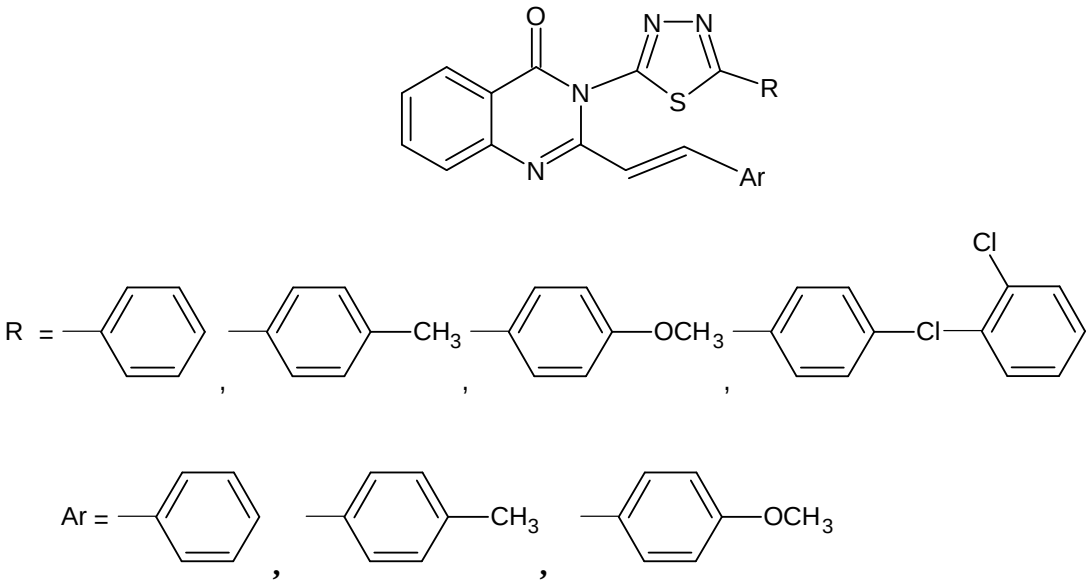


Rajiv Dahiya *et al.*, (2008) synthesized peptide derivatives of iodoquinazolinones, nitroimidazoles and screened for antimicrobial and antihelmintic activities.

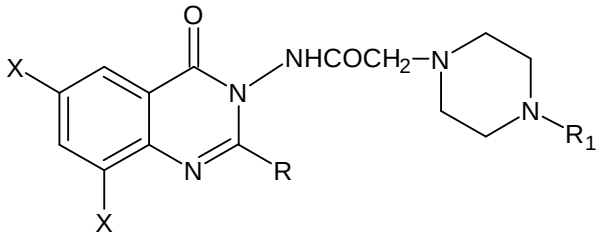


X= Val-Tyr-Phe-Gly

Varsha Jatav *et al.*, (2008) synthesized some novel 3-[5-substituted 1, 3, 4-thiadiazole-2-yl]-2-styryl quinazolin-4(3H) ones exhibiting significant anticonvulsant, sedative-hypnotic and CNS depressant activities.



Ragahavendra N. M. *et al.*, (2008) synthesized some novel substituted piperazinyl quinazolin-3(4H)-ones and evaluated for antibacterial and antifungal activity.

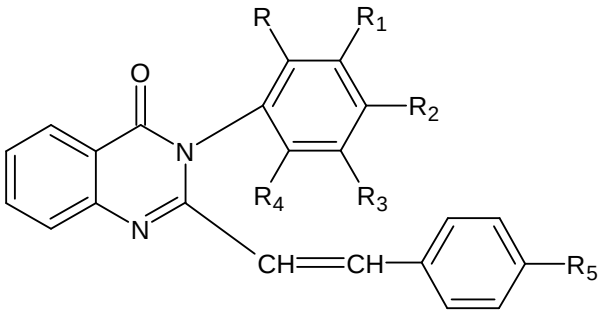


X = H, Br

R = C_6H_5, C_3H_7, CH_3

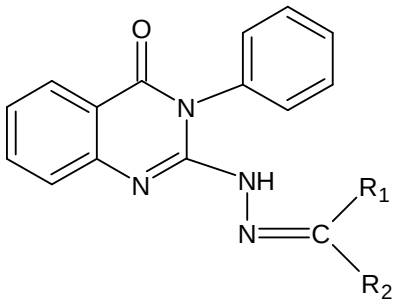
R_1 = CH_3,C_2H_5.

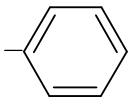
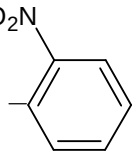
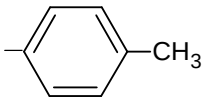
Jessy E. M. *et al.*, (2007) synthesized a series of novel 2,3-disubstituted 3,1-quinazolin-4-(3H)-ones showing good antibacterial and antiinflammatory activities.

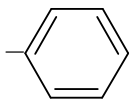


R, R_1= H, COOH R_2 = H, Br R_3, R_4 = H, Cl R_5= NO_2, OCH_3, F, OH.

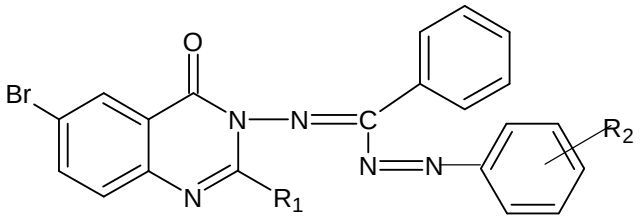
Alagarsamy V. *et al.*, (2007) synthesized some 3-phenyl-2-substituted-3H-quinazolin-4-ones for analgesic and antiinflammatory activities.



R_1= CH_3, C_2H_5,  ,  , 

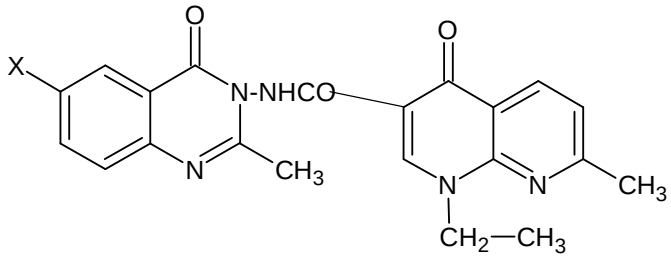
R_2= H, C_2H_5, C_3H_7, 

Desai K. R. *et al.*, (2006) synthesized 6-bromo-2-alkyl/aryl-3-{[phenyl (phenyl diazenyl) methylene] amino} quinazolin-4(3H) one for antibacterial activity.



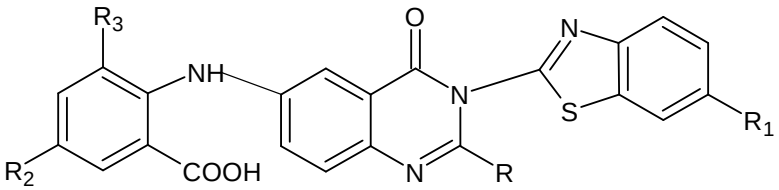
$R_1 = CH_3$ $R_2 =$ H, 2-OCH_3, 3-OCH_3, 2-Br, 3-Br, 2-Cl, 3-Cl, 2-NO_2, 3-NO_2, 2-CH_3, 3-CH_3.

Gaurav Grover *et al.*, (2006) synthesized some new quinazolone derivatives of nalidixic acid as potential antibacterial and antifungal agents.



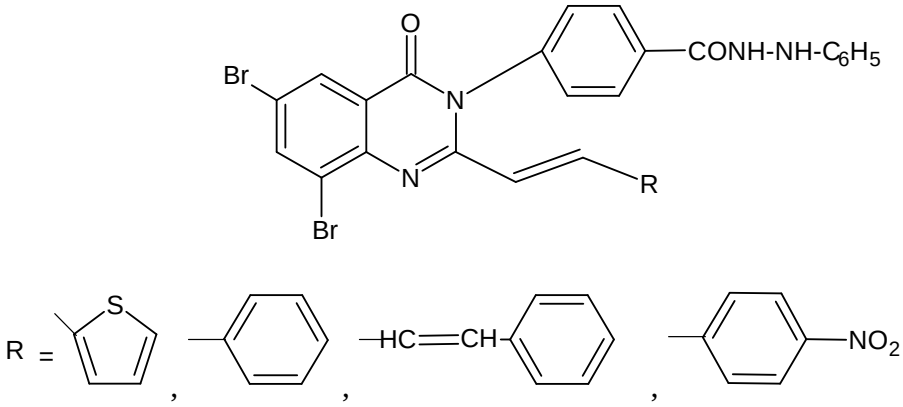
X= H, I, NO_2.

Manish P Patel *et al.*, (2005) synthesized some new 2, 3, 6-trisubstituted quinazolin-4(3H)-ones and screened for antibacterial activity.

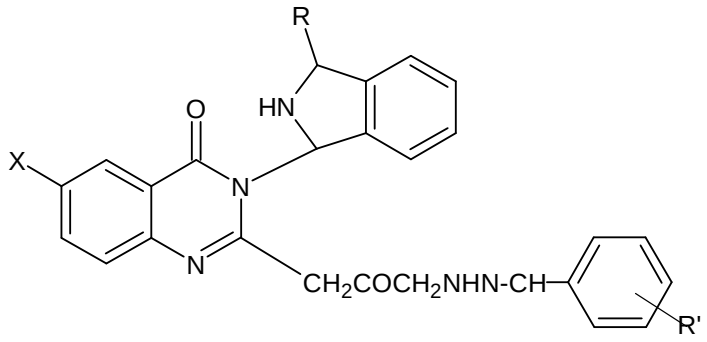


R= C_6H_5, CH_3 R_1= OCH_3 R_2, R3= Br, H.

Murugan V. *et al.*, (2003) synthesized 6, 8-dibromo-2-substituted styryl-4-quinazolin-3(4-benzphenylhydrazides) showing significant anticancer activity.

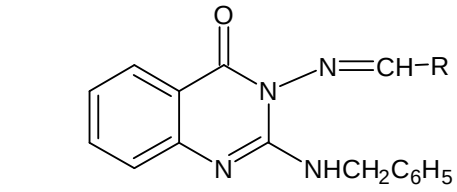


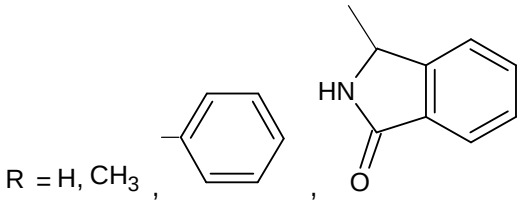
Ashok Kumar *et al.*, (2003) synthesized some new 2, 3, 6-trisubstituted quinazolinones and evaluated for their antiinflammatory, analgesic and COX-II inhibiting properties.



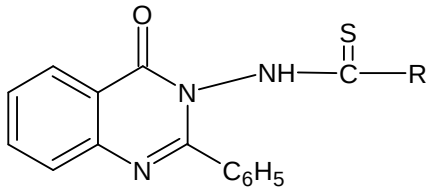
X = H, I, Br, R= H, CH_3, R'= 2-OCH_3, 4-OCH_3, 2-Cl, 4-Cl.

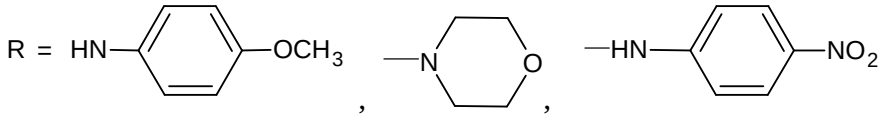
Alagarsamy V. *et al.*, (2003) synthesized a series of novel 2-benzylamino-3-substituted quinazolin-4-(3H)-ones studied for analgesic and antiinflammatory activities.



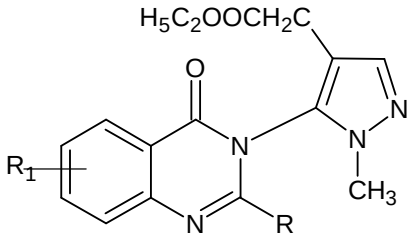


Alagarsamy V. *et al.*, (2002) synthesized some novel 2-phenyl-3-substituted quinazolin-4(3H) ones derivatives and performed for analgesic and antiinflammatory activities.





Benedetta Maggio *et al.*, (2001) synthesized some new ethyl 1-methyl-5-(substituted 3, 4-dihydro-4-oxoquinazolin-3-yl)-1H-pyrazole-4-acetates and showing appreciable analgesic, antiinflammatoy and ulcerogenic activities.



R = H, CH_3, C_2H_5, C_6H_5 R_1= 6-Cl, 7-Cl, 8-Cl.

EXPERIMENTAL SECTION

IV. EXPERIMENTAL SECTION

A. MATERIALS AND METHODS

List of Chemicals and Instruments used for the study:

Table-1

S. No	Chemicals / Instruments	Supplier / Model
	Chemicals	
1	Glycine	Lobachem Pvt. Ltd.,
2	Alanine	Lobachem Pvt. Ltd.,
3	Phenyl alanine	Lobachem Pvt. Ltd.,
4	Leucine	Lobachem Pvt. Ltd.,
5	Valine	Lobachem Pvt. Ltd.,
6	Anthranilic acid	Sigma Aldrich
7	Acetic anhydride	Lobachem Pvt. Ltd.,
8	Glacial acetic acid	Spectrochem Pvt. Ltd.,
9	p-Hydroxy benzaldehyde	Lobachem Pvt. Ltd.,
10	p-Dimethyl amino benzaldehyde	Lobachem Pvt. Ltd.,
11	p-Methoxy benzaldehyde	Lobachem Pvt. Ltd.,
12	p-Nitro benzaldehyde	Lobachem Pvt. Ltd.,
13	t-butyloxy carbonic anhydride	Lobachem Pvt. Ltd.,
14	Isopropyl alcohol	Lobachem Pvt. Ltd.,
15	Chloroform	Spectrochem Pvt. Ltd.,
16	Petroleum ether (40-60^{0}C)	Qualigens Fine Chemicals

17	Dichloromethane	Lobachem Pvt. Ltd.,
18	Methanol	Qualigens fine Chemicals
19	Ethanol	Lobachem Pvt. Ltd.,
20	Thionyl chloride	Lobachem Pvt. Ltd.,
21	Triethylamine	Qualigens Fine Chemicals
22	EDC (1-ethyl-3(3-dimethyl aminopropyl) carbodimide hydrochloride)	Spectrochem Pvt. Ltd.,
23	Trifluroacetic acid	Qualigens Fine Chemicals
24	Sodium hydroxide	Spectrochem Pvt. Ltd.,
25	Anhydrous sodium sulphate	Qualigens Fine Chemicals
26	Sodium bicarbonate	Lobachem Pvt. Ltd.,
27	Sodium chloride	Lobachem Pvt. Ltd.,
28	Diethyl ether	Lobachem Pvt. Ltd.,
29	Conc. sulphuric acid	Qualigens Fine Chemicals
30	Hexane	Merck Pvt. Ltd.,
31	Ethyl acetate	Lobachem Pvt. Ltd.,
32	Silica Gel	Lobachem Pvt. Ltd.,
	Instruments	
33	Magnetic stirrer	Remi Equipments
34	Eddy's hot plate	Instrumental and chemical Pvt. Ltd

35	Weighing balance	Shzimadzhu 220 v
36	Melting point apparatus	Sun bim Equipments
37	Hot air oven	Picses Instruments
38	Heating mantle	Ajay Thermo Electris

Methods

- The solution phase technique was followed to synthesize the dipeptide derivatives.
- The melting point was determined using an open ended capillary tube method.
- The completion of the reaction was checked by thin layer chromatography using silica gel G as

  stationary phase.
- The IR spectrum of the synthesized compounds was recorded in JASCO FT-IR spectrophotometer, Ideal analytical and research institution, Puducherry.
- The ^{1}H NMR spectrum of the synthesized compounds was recorded in BRUKER 500 MHz NMR spectrophotometer, SAIF, IIT-Madras.
- The mass spectrum of the synthesized compounds was recorded in JOEL GCmate by electron impact method as ionization mode, SAIF, IIT-Madras.
- The analgesic activity of the synthesized compounds was screened by Eddy's Hot plate method.
- The antibacterial and antifungal activity was performed by disc diffusion method in Pharma analytical laboratory, Puducherry.

B. SCHEME OF THE WORK

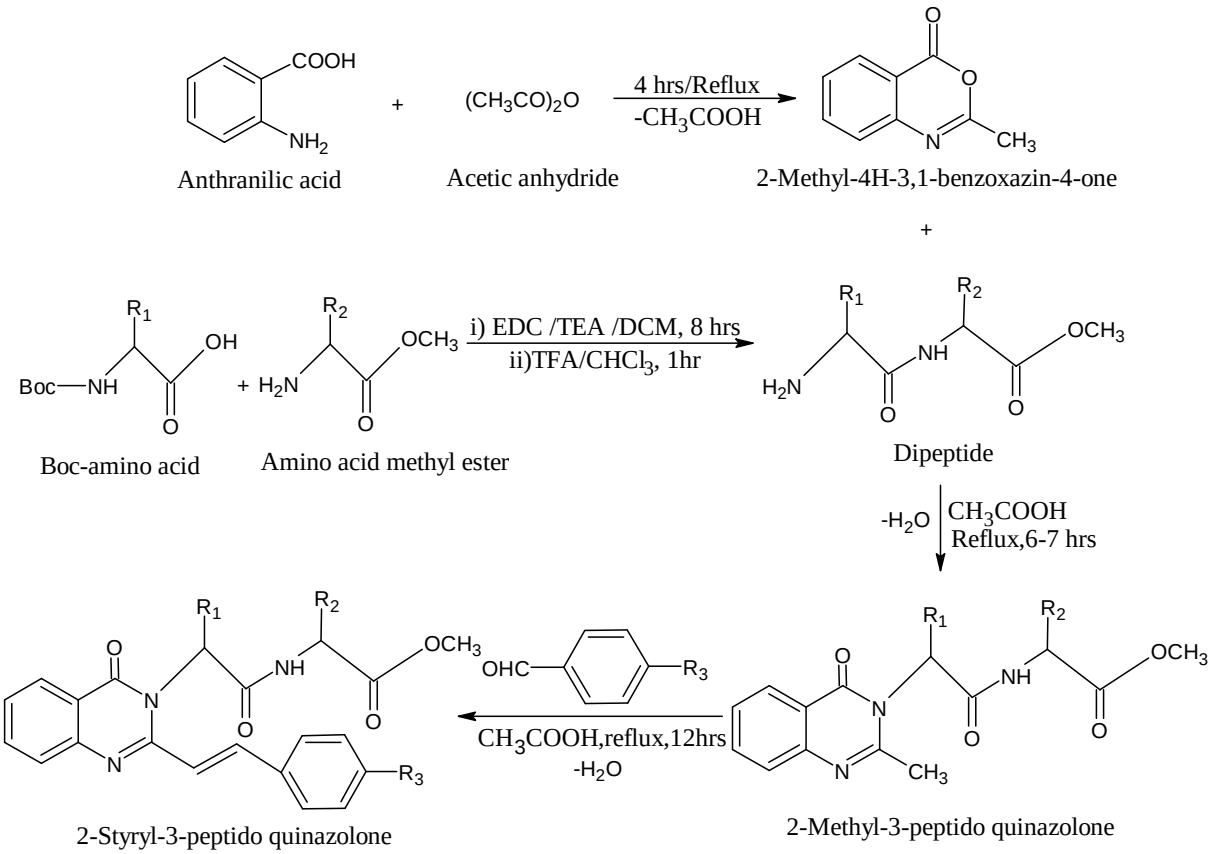


Figure.1

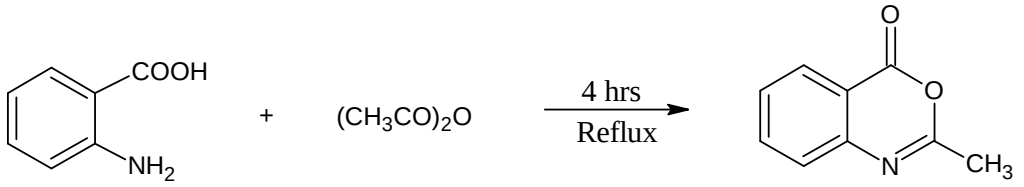
Compound	R_1	R_2	R_3
QZ-1	-H	$-CH_2C_6H_5$	-OH
QZ-2	$-CH_2-CH(CH_3)_2$	$-CH_2C_6H_5$	-OH
QZ-3	$-CH_2-CH(CH_3)_2$	$-CH_2C_6H_5$	$-N(CH_3)_2$
QZ-4	$-CH(CH_3)_2$	$-CH_2-CH(CH_3)_2$	$-NO_2$
QZ-5	$-CH_3$	$-CH_2-CH(CH_3)_2$	$-OCH_3$
QZ-6	$-CH_3$	$-CH_2-CH(CH_3)_2$	$-NO_2$

C. METHODOLOGY

SYNTHESIS OF QUINAZOLONE DERIVATIVE

1. Synthesis of 2-methyl-4H-3,1-benzoxazin-4-one

Anthranilic acid (0.12 mol) and acetic anhydride (0.2 mol) was refluxed for 4 hrs under anhydrous condition and the excess of acetic anhydride was removed under pressure. Cooled to room temperature and the product was immediately used for the next step due to its instability for longer time. (*Jessy E.M. et al., 2007*)

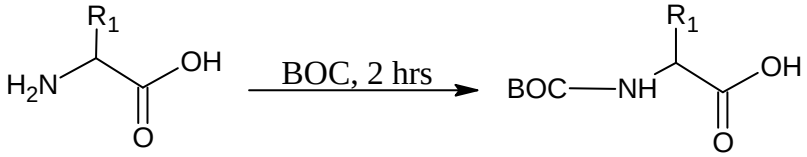


Anthranilic acid Acetic anhydride 2-Methyl-4H-3,1-benzoxazin-4-one

2. Preparation of peptides

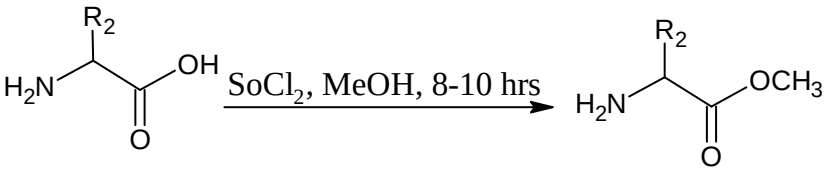
i) Protection of Amino Group

The amino acid (20 m.mol) was dissolved in a mixture of 1N NaOH (20 ml) and IPA (20 ml). To this BOC (26 m.mol, 6 ml) in IPA (10 ml) was added, followed by 1N NaOH (20 ml) to the resulting solution. The solution was stirred at room temperature for 2 hrs, washed with light petroleum ether (40 – 60^0C) (20 ml), acidified to pH-3 with 2N sulphuric acid and finally extracted with chloroform (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under pressure to give the BOC-amino acid. The crude product was recrystallized using a mixture of chloroform and petroleum ether. (*Bodanszky. M., 1984)*



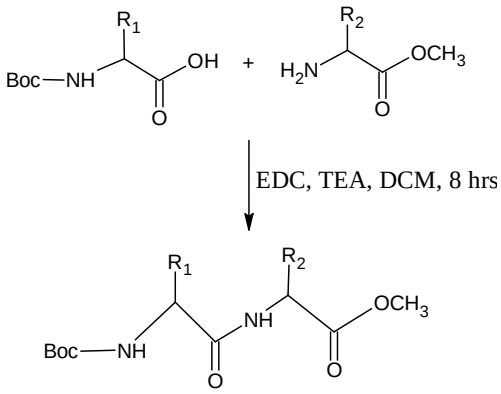
ii) Protection of Carboxylic Group

Thionyl chloride (20 m.mol) was added to methanol (100 ml) slowly at 0°C and the amino acid was added to this solution and it was refluxed for 8 to 10 hrs. The solvent was evaporated to give the amino acid methyl ester hydrochloride, which was triturated with ether at 0°C until the excess dimethyl sulphite was removed. The resulting solution was recrystallized from methanol and diethyl ether at 0°C. (*Bodanszky. E., 1984*)



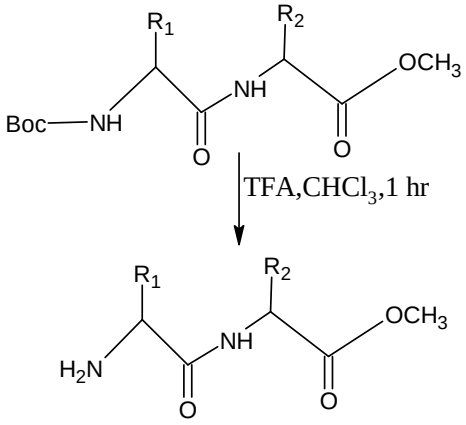
iii) Synthesis of Dipeptides

Amino acid methyl ester hydrochloride (10 m.mol) was dissolved in DCM (20 ml). To this triethyl amine (4 ml) was added at 0°C and the reaction mixture was stirred for 15 mins. BOC- amino acid (10 m.mol) in chloroform (20 ml) and EDC (10 m.mol) were added with stirring. After 8 hrs the reaction mixture was filtered and residue was washed with chloroform (30 ml) and added to the filtrate. The filtrate was washed with 5% sodium bicarbonate (20 ml) and saturated sodium chloride (20 ml) and water. Organic layer was dried over anhydrous sodium sulphate and the product was recrystallized by chloroform and petroleum ether. (*Bodanszky. M., 1984*)



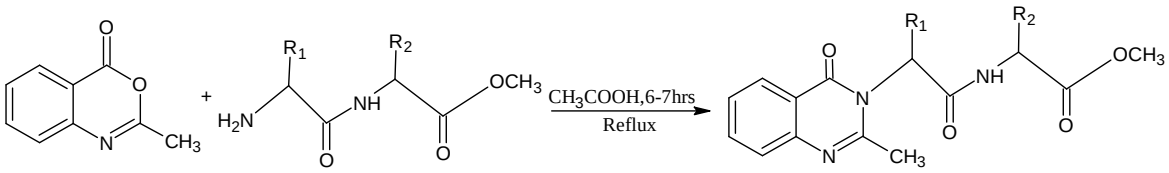
iv) Deprotection of Amino Group

The protected peptide was dissolved in chloroform (15 ml) and treated with trifluro acetic acid (2 m.mol). The solution was stirred at room temp for 1hour and washed with saturated sodium bicarbonate (5 ml) and the organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. (*Bodanszky. M., 1984)*



3. Synthesis of 2-methyl-3-peptido quinazolin-4-(3H)-ones

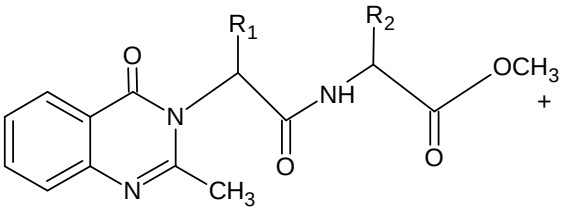
An equimolar mixture of 2-methyl-4H-3,1-benzoxazin-4-one (0.01 mol) was reacted with deprotected dipeptide (0.01 mol) having free terminal amino group and protected carboxylic terminal was refluxed for 6-7 hours in the presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature and poured into crushed ice; the crude product obtained was recrystallized from ethanol. *(Jessy E.M. et al., 2007)*



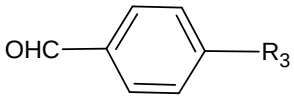
2-Methyl-4H-3,1-benzoxazin-4-one Dipeptide 2-Methyl-3-Peptido quinazolin-4-(3H)-ones

4. Synthesis of 2-styryl-3-peptido quinazolin-4-(3H) ones:

A mixture of quinazolinone (0.01 mol) and various 4-substituted benzaldehyde (0.01 mol) was reacted with glacial acetic acid (50 ml) and refluxed for 12 hours. The reaction mixture was poured into ice cold water, filtered, dried and recrytallized from ethanol. *(Varsha Jatav et al., 2008)*

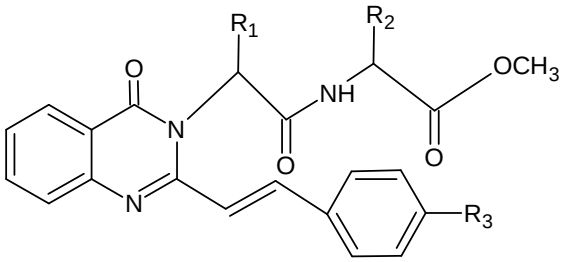


2-Methyl-3-Peptido quinazolin-4-(3H)-ones



Aromatic aldehyde

CH_3COOH,12hrs Reflux



2-Styryl-3-peptido quinazolin-4-(3H)-ones

D. EVALUATION OF ANALGESIC ACTIVITY

Acute pain is generally well accounted for interms of nociception, on excessive noxious stimulus giving risk to an intense and unpleaseant sensation. In general depending upon nature of stimulus, physical, thermal and chemical methods are employed for evaluation of analgesic property of the compound. The analgesic activity are usually carried out as follows

Physical stimulus

Tail-clip method

Thermal stimulus

Hot plate method

Tail-flick response

Tail-immersion method

Chemical stimulus

Writhing test

Writhing induced by 4% sodium chloride solution

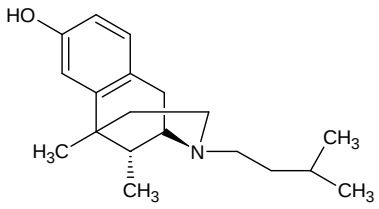
Writhing induced by Aconitine

The thermal stimulus method was followed to screen the analgesic activity. In this method the pain is induced by heat from Eddy's Hot plate.

Standard drug for Analgesic Activity

To screen and compare the analgesic activity of synthesized compounds the pentazocine was used as a standard drug.

Structure



Pentazocine

Pentazocine is an opioid agonist-antagonist. It has agonistic action at κ and weak antagonistic action at μ receptors.

Analgesic activity: Eddy's Hot Plate Method

The analgesic activity was evaluated by Eddy's hot plate method using pentazocine as standard. Albino mice of either sex (20 - 25g) were divided into control, standard and test each with six animals. The animals were acclimated to laboratory conditions for one week prior to the experiment. They were subjected to standard diet and water *ad libitum*, but 12 hrs prior to an experiment the rats were deprived of food but not water. The animal study protocol was approved by CPCSEA (Reg No: 409/01/CPCSEA). The test compounds were administered intraperitonially with a dose of 50 mg/kg in 1% polyethylene glycol.

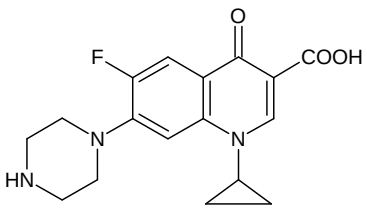
The control group was given only 1% v/v polyethylene glycol. Standard group was administered with pentazocine as standard intraperitonially in a dose of 20mg/kg. Mice were placed on the hot plate maintaining at 55°C ± 1°C and the reaction time was noted in seconds that is licking of paw or jumping response. The cut off time is 15 seconds and the animals not showing any response after 15 seconds are removed from the study. The reaction time was

measured before and after administration of the compounds at the interval of 0, 30, 60, 90 and 180 minutes. (*Eddy N. B., et al., 1953*)

E. EVALUATION OF ANTIBACTERIAL ACTIVITY

Microbiological assay is defined as the *determination or estimation of* **concentration or potency** of an **antibiotic** by means of **measuring and comparing** the *area of zone of inhibition in mm or turbidity produced* by test substance with that of standard over a suitable microbe under standard conditions.

Standard drug for Antibacterial Activity



Ciprofloxacin

Ciprofloxacin is a broad spectrum antibiotic

Organism used

Gram positive Organisms

Micrococlus luteus ATCC 9341

Gram negative Organisms

Klebsiella pneumoniae ATCC 29665

The antibacterial activity of synthesized compounds were screened in the concentration of 50, 100, and 150 µg/ml in dimethyl formamide against gram positive *Micrococlus luteus* and gram negative *Klebsiella pneumoniae* in Muller Hinton agar medium by disc diffussion method.

Preparation of Muller Hinton Agar

Muller Hinton agar is considered the best medium to use for routine susceptibility testing of non fastidious bacteria and it shows acceptable batch-to-batch reproducibility for susceptibility testing.

Composition

Beef extract	10.0 grams
Casein acid hydrolysis	17.5 grams
Starch	1.50 grams
Agar	20.0 grams
Distilled water	1000 ml

All the ingredients were taken in 1000 ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was adjusted to 7 ± 0.2 and sterilized in autoclave at 15 lb at 120^0C for 15 minutes. The sterile medium was poured into petri dish and allowed to solidify.

Preparation of the Disks

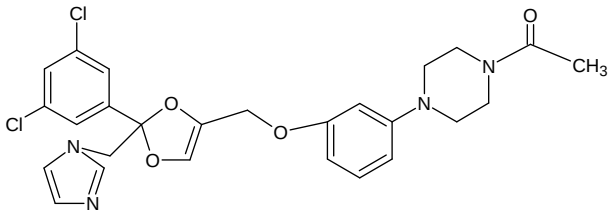
Paper disks of 6 mm diameter and 2 mm thickness were sterilized by autoclaving at 121^0C for 15 minutes. Ciprofloxacin (10 µg/ml disk) was used as standard antibiotic for the comparison of antibacterial activity of the synthesized compounds.

Disc Diffusion method

Whatmann filter paper grade-1 disc of 5 mm diameter and 2 mm width was sterilized by autoclaving for 15 min at 121°C. The sterile discs were impregnated with different synthesized compounds which were dissolved in dimethyl formamide. The steriled agar petridish was seeded with test bacteria and the impregnated discs were placed on the medium with suitable space between the discs. The plates were incubated at 37°C ± 1°C for 18-24 hrs for bacterial medium. The inhibition of zones caused by the synthesized compounds and standard drug were examined and the diameter of zone of inhibition was observed and recorded.

F. EVALUATION OF ANTIFUNGAL ACTIVITY

Standard drug for Antifungal Activity



Ketaconazole

It is broad spectrum antifungal agent.

Organism used

Candida albicans ATCC 10231

The antifungal activity of synthesized compounds were screened in the concentration of 50, 100, and 150 µg/ml disc in dimethyl formamide against *Candida albicans* in Sabouraud's agar medium by disc diffussion method.

Preparation of Sabouraud's Agar Media

Composition

Dextrose	20 gms
Peptone	10 gms
Purified water	1000 ml
pH	5.4± 0.2
Agar	15 gm

The media was prepared by dissolving the specified quantities of the dehydrated ingredients (Hi-media) in purified water and was distributed in Petri dish to a thickness of 3-4 mm. The plates sterilized by autoclaving at 121^0C for 15 minutes.

Disc diffusion method

Whatmann filter paper grade-1 disc of 5 mm diameter and 2 mm width was sterilized by autoclaving for 15 min at 121°C. The sterile discs were impregnated with different synthesized compounds which were dissolved in dimethyl formamide. The steriled agar petridish was seeded with test bacteria and the impregnated discs were placed on the medium with suitable space between the discs. The plates were incubated at 25°C ± 1°C for 72 hrs for fungal medium. The inhibition of zones caused by various synthesized compounds and standard drug were examined and the diameter of zone of inhibition was observed and recoded.

RESULTS AND DISCUSSION

V. RESULTS AND DISCUSSION

The solution phase technique was employed to synthesize the dipeptides. Dipeptides were prepared by coupling Boc-amino acids with the amino acid methyl ester hydrochloride using EDC as coupling agent. All the dipeptides were deprotected at the amino end using trifluoroacetic acid. 2-methyl-4H-3,1-benzoxazin-4-one was made to react with dipeptides to get a peptido quinazolones. These compounds were further condensed with different substituted aromatic aldehydes to get 2-styryl-3-peptido quinazolones. The melting point was determined using an open ended capillary tube method and the completion of reaction was checked by thin layer chromatography using silica gel as stationary phase and hexane:ethyl acetate (8:2) as mobile phase. The spot in the TLC plate was detected by iodine vapours. The chemical structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral data.

A. PHYSICAL DATA OF THE COMPOUNDS

i) Phyiscal data of Boc-amino acid

The amino group of amino acids was protected by t-butyloxy carbonic anhydride (Boc). The Boc amino acids were obtained with good yield. The physical data of the Boc- amino acids are given below.

Table-2

S. No	Boc-amino acid	Molecular Formula	Molecular weight	Physical state	Melting Point (C°)	Yield (%)	R_f^* Value
1.	Boc-Gly	$C_7H_{13}NO_4$	175.18	Semisolid	86-89	66.31	0.75
2.	Boc-Ala	$C_8H_{15}NO_4$	189.21	White powder	79-83	81.46	0.54
3.	Boc-Leu	$C_{11}H_{21}NO_4$	231.29	White powder	82-85	74.13	0.45
4.	Boc-Val	$C_{10}H_{19}NO_4$	217.26	White powder	76-80	77.36	0.80

* Solvent system: Chloroform: Methanol (7:3)

ii) Physical data of amino acid methyl ester Hydrochloride

The carboxylic group of amino acids was protected by methanol and thionyl chloride mixture and the products were obtained with good yield. The physical data of the amino acid methyl esters are given below.

Table-3

S. No	Amino acid methyl ester	Molecular formula	Molecular Weight	Physical state	Melting Point(C°)	Yield (%)	R_f^* Value
1.	Phe-OMe. HCl	$C_{10}H_{14}ClNO_2$	215.69	White powder	142-146	98.83	0.41
2.	Leu-OMe. HCl	$C_7H_{16}ClNO_2$	181.66	White powder	140	74.08	0.77

* Solvent system: Chloroform: Methanol (7:3)

iii) Physical data of dipeptides

The Boc amino acid and the amino acid methyl ester hydrochloride were coupled using EDC as coupling agent. The dipeptide was obtained with good yield. The physical data of dipeptides are given below.

Table-4

S. No	Dipeptides	Molecular formula	Molecular weight	Physical state	Yield (%)	R_f* Value
1.	Boc-Gly-Phe-OCH_3.HCl	$C_{17}H_{25}N_2O_5Cl$	372	Semisolid	77.63	0.55
2.	Boc-Ala-Leu-OCH_3.HCl	$C_{15}H_{29}N_2O_5Cl$	352	Semisolid	62.58	0.57
3.	Boc-Leu-Phe-OCH_3.HCl	$C_{21}H_{33}N_2O_5Cl$	428	Semisolid	71.39	0.58
4.	Boc-Val-Leu-OCH_3.HCl	$C_{17}H_{33}N_2O_5Cl$	380	Semisolid	65.85	0.53

* Solvent system: Hexane: Ethyl acetate (8:2).

iv) Physical data of amino group deprotected dipeptides

The Boc protected amino groups was deprotected using trifluroacetic acid in chloroform and the physical data of amino group deprotected dipeptides are given below.

Table-5

S. No	Dipeptides	Molecular formula	Molecular weight	Physical state	Yield (%)	R_f* Value
1.	H_2N-Gly-Phe-OCH_3	$C_{12}H_{16}N_2O_3$	236	Semisolid	75.54	0.54
2.	H_2N-Ala-Leu-OCH_3	$C_{10}H_{20}N_2O_3$	216	Semisolid	79.13	0.51
3.	H_2N-Leu-Phe-OCH_3	$C_{16}H_{24}N_2O_3$	292	Semisolid	68.43	0.71
4.	H_2N-Val-Leu-OCH_3	$C_{12}H_{24}N_2O_3$	244	Semisolid	88.48	0.67

* Solvent system: Hexane: Ethyl acetate (8:2).

v) Physical data of 2-methyl-3-peptido quinazolones

The dipeptides having free terminal amino group and protected carboxylic terminal are incorporated in 2-methyl-4H-3,1 benzoxazin-4-one to get peptido quinazolones. The physical data of 2-methyl-3-quinazolone are given below.

Table-6

Compound	**Molecular Formula**	**Molecular weight**	**Colour**	**Melting point (C°)**	**Yield (%)**	**R_f* Value**
MQZ-I	$C_{25}H_{29}N_3O_4$	435.51	Pale yellow	162	86.84	0.64
MQZ-2	$C_{19}H_{25}N_3O_4$	359.41	Light brown	172	55.92	0.58
MQZ-3	$C_{21}H_{29}N_3O_4$	387.47	Dark brown	234	68.56	0.78
MQZ-4	$C_{21}H_{21}N_3O_4$	379.40	Pale yellow	142	65.98	0.54

*Solvent system: Hexane: Ethyl acetate (8:2)

vi) Physical data of 2-styryl-3-peptido quinazolones

The 2-methyl-3-peptido quinazolone was condensed with different substituted aromatic aldehydes to get 2-styryl 3- peptido quinazolones. The physical data of 2-styryl-3-peptido quinazolone are given below.

Table-7

Compound	Molecular formula	Molecular weight	Colour	Melting Point(C°)	Yield (%)	R_f^* Value
QZ-1	$C_{28}H_{25}N_3O_5$	483	Brownish black	252	64.33	0.58
QZ-2	$C_{32}H_{33}N_3O_5$	539	Light orange	226	66.20	0.69
QZ-3	$C_{34}H_{38}N_4O_4$	566	Light brown	187	56.38	0.41
QZ-4	$C_{28}H_{32}N_4O_6$	520	Pale yellow	192	63.52	0.59
QZ-5	$C_{26}H_{28}N_4O_6$	492	Reddish brown	153	60.87	0.46
QZ-6	$C_{27}H_{31}N_3O_5$	477	Dark brown	264	52.94	0.41

*Solvent system: Hexane: Ethyl acetate (8:2).

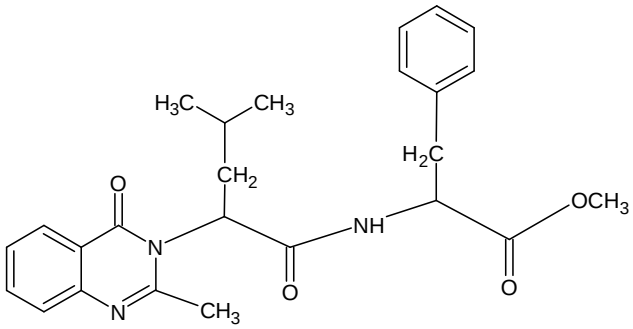
B. SPECTRAL ANALYSIS

The synthesized compounds were characterized by IR, proton NMR and Mass spectrophotometer and the structures of the compounds are consistent with the spectra.

i) IR Spectral Analysis

The IR spectrum was recorded in JASCO FT-IR spectrophotometer. The signifigant IR values are measured in cm^{-1} and the results given in the table.

Interpretation of IR spectra of MQZ-1



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-8

S. No	Wave numbers (cm^{-1})	Functional groups
1.	3476	N-H Stretching in amide
2.	2959	C-H Stretching in CH_3
3.	2854	-CH_2- Stretching
4.	1701	C=O Stretching in amide
5.	1605	Aromatic C=C Stretching
6.	1453	C=N Stretching

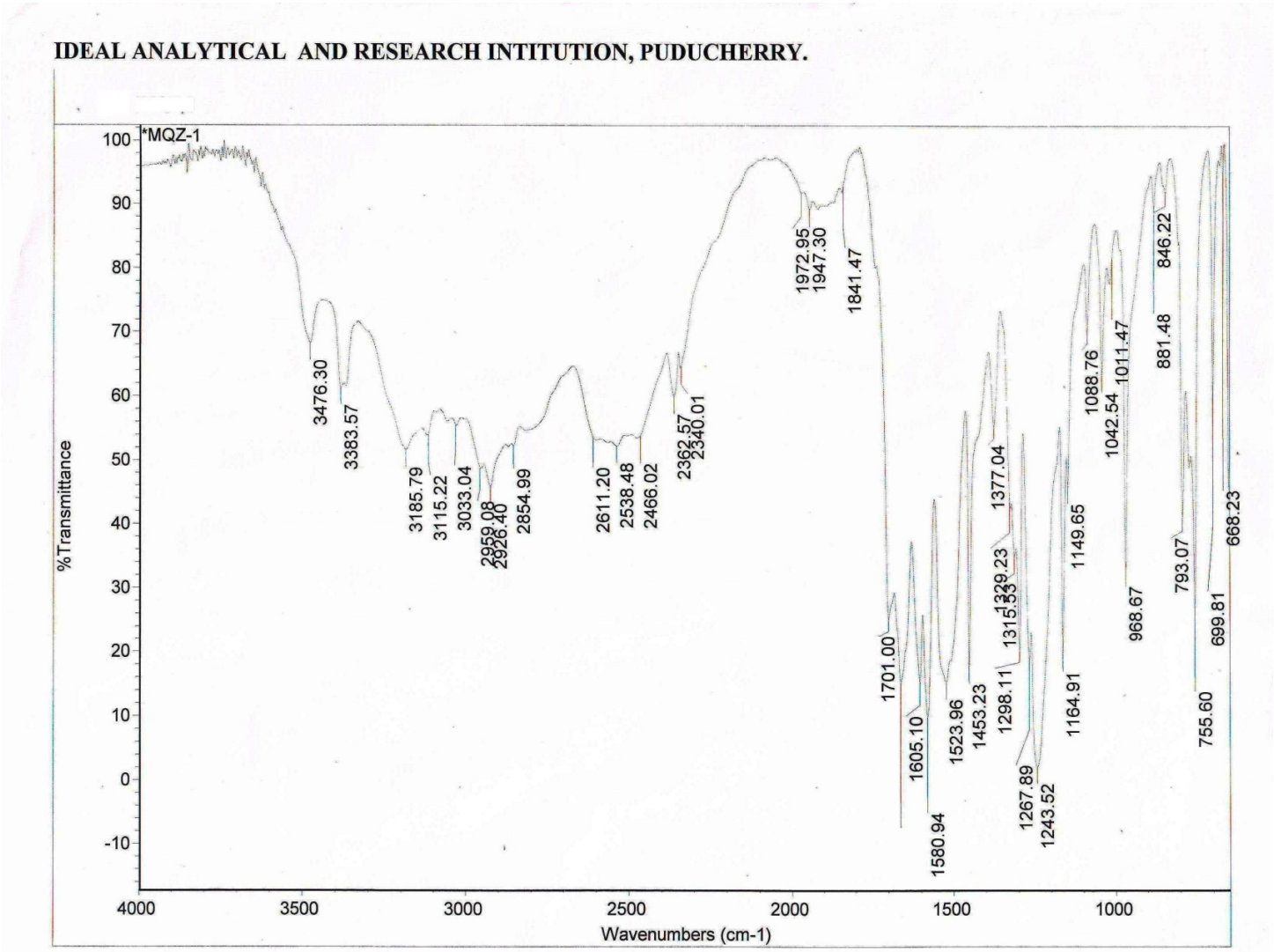
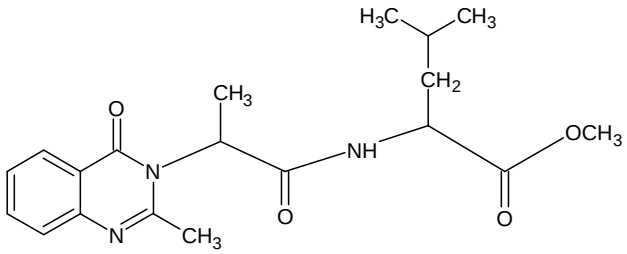


Figure 2: IR Spectra of MQZ-1

Interpretation of IR spectra of MQZ-2



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-9

S. No	Wave numbers (cm^{-1})	Functional groups
1.	3386	N-H Stretching in amide
2.	2925	C-H Stretching in CH_3
3.	2854	-CH_2- Stretching
4.	1700	C=O Stretching in amide
5.	1654	Aromatic C=C Stretching
6.	1453	C=N Stretching

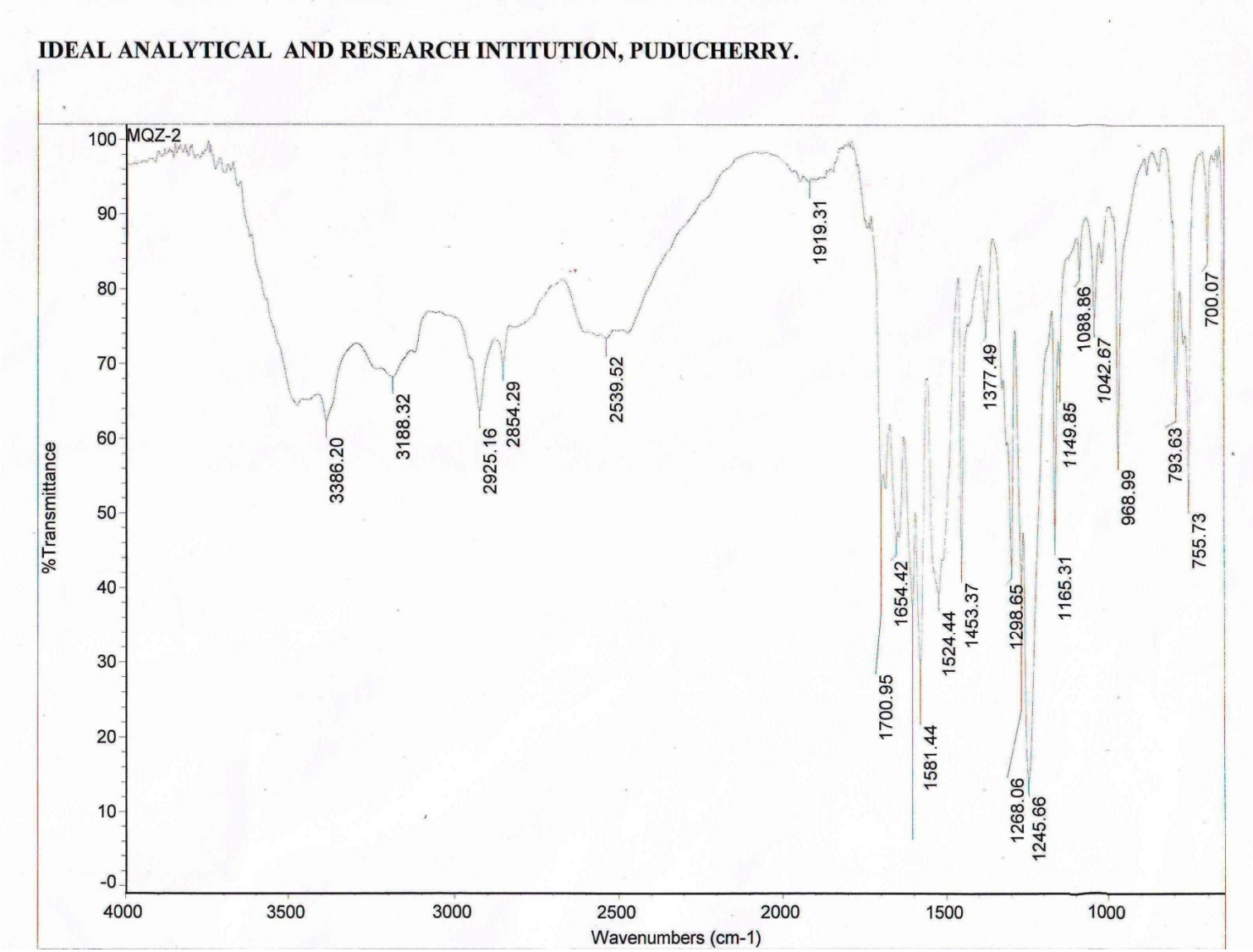
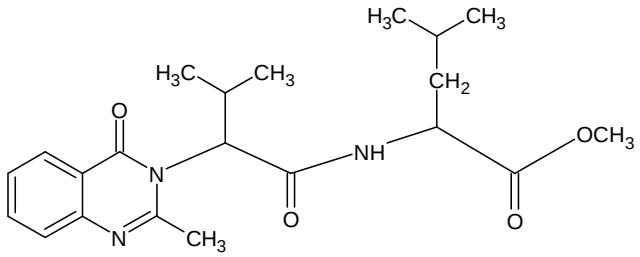


Figure 3: IR Spectra of MQZ-2

Interpretation of IR spectra of MQZ-3



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-10

S. No	Wave numbers (cm^{-1})	Functional groups
1.	3385	N-H Stretching
2.	2924	C-H Stretching in CH_3
3.	2854	-CH_2- Stretching
4.	1737	C=O Stretching in amide
5.	1655	Aromatic C=C Stretching
6.	1377	C-H bending

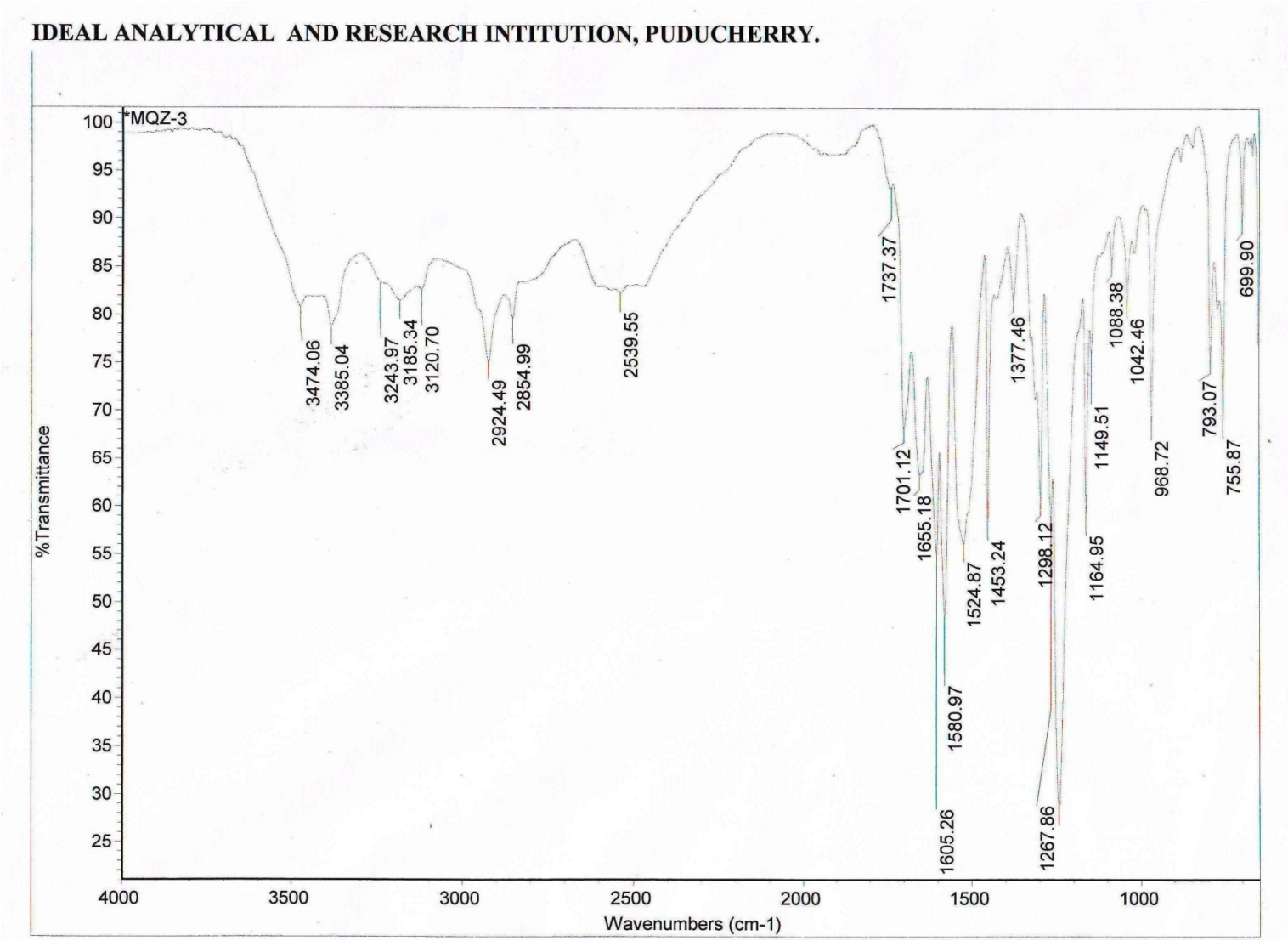
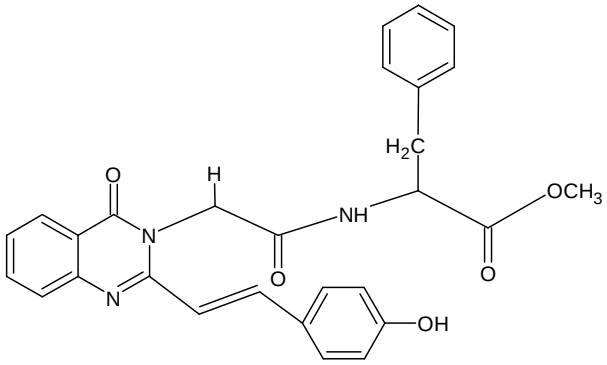


Figure 4: IR Spectra of MQZ-3

Interpretation of IR Spectra of QZ-1



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-11

S. No	Wave numbers (cm^{-1})	Functional groups
1.	3430	NH Stretching in amide
2.	3211	Aromatic-OH Stretching
3.	2924	$-CH_2-$ Stretching
4.	1745	C=O Stretching in amide
5.	1665	Aromatic C=C Stretching
6.	966	Olefinic CH=CH Stretching

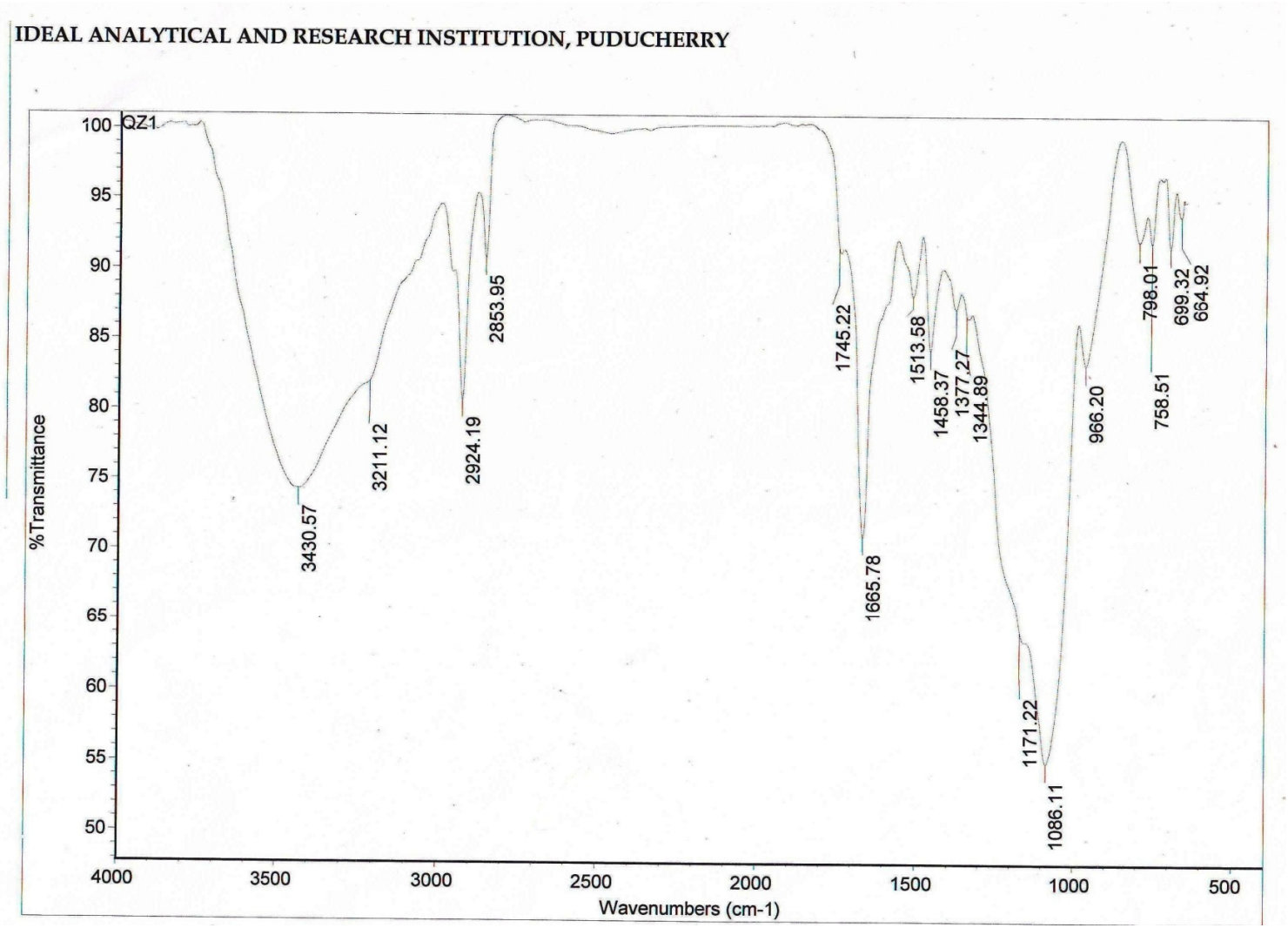
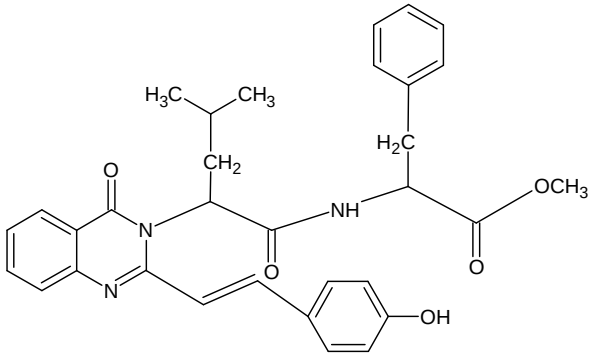


Figure 5: IR Spectra of QZ-1

Interpretation of IR Spectra of QZ-2



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-12

S. No	Wave numbers (cm^{-1})	Functional groups
1.	3442	NH Stretching in amide
2.	3378	Aromatic-OH Stretching
3.	2854	$-CH_2-$ Stretching
4.	1582	Aromatic C=C Stretching
5.	1664	C=O Stretching in amide
6.	966	Olefinic CH=CH Stretching

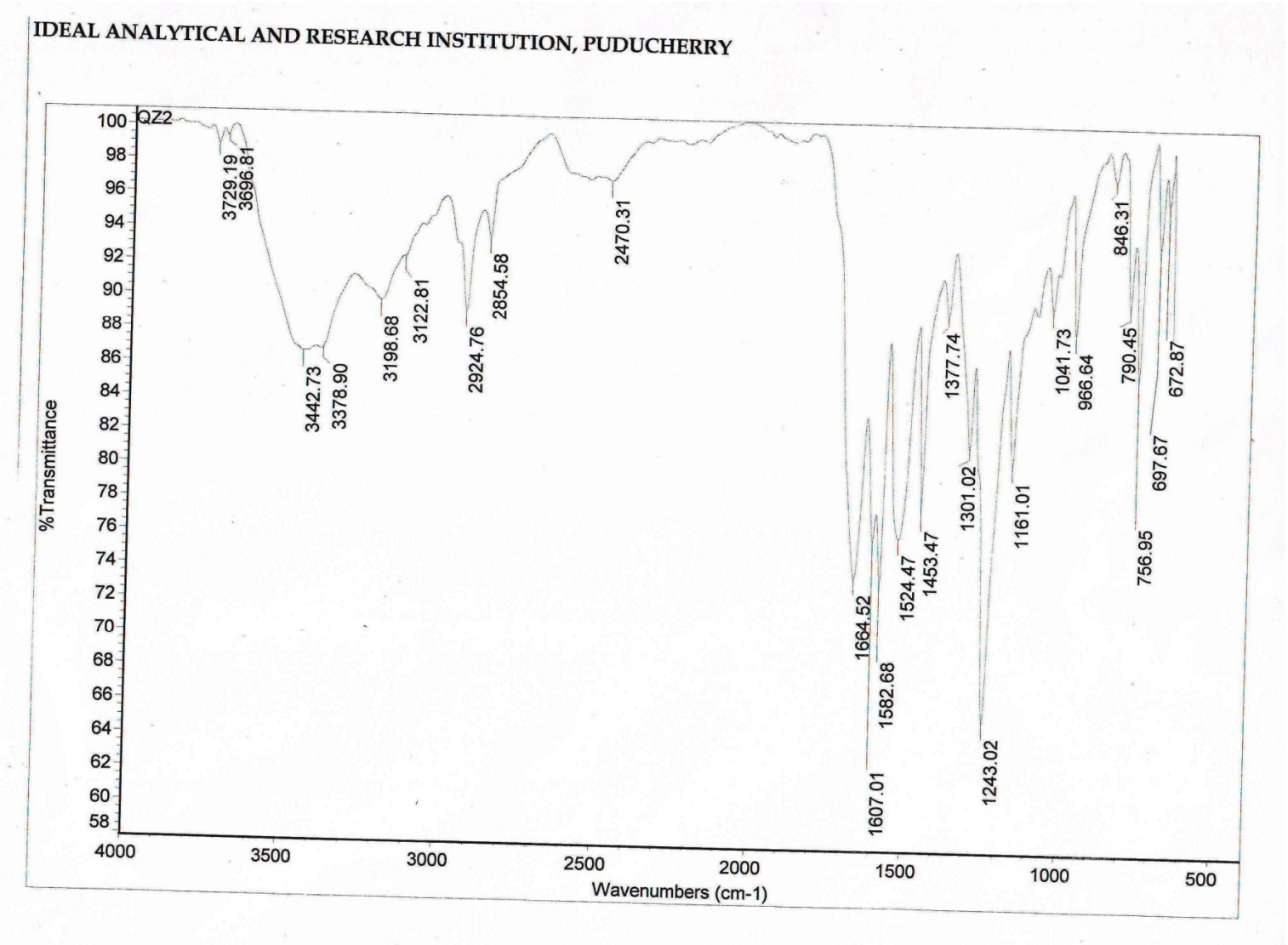
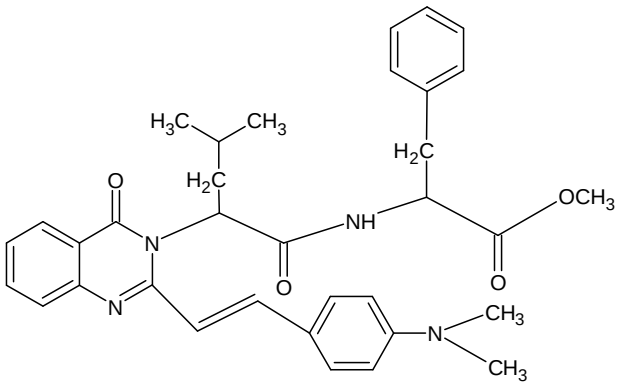


Figure 6: IR Spectra of QZ-2

Interpretation of IR Spectra of QZ-3



The significant wave numbers of the compound and its relevant functional groups are given below:

Table-13

S. No	Wave numbers (cm^{-1})	Functional groups
1.	3443	NH Stretching in amide
2.	3030	-CH_2- Stretching
3.	1587	Aromatic C=C Stretching
4.	1669	C=O Stretching in amide
5.	1300	(N-methyl) Stretching
6.	966	Olefinic CH=CH Stretching

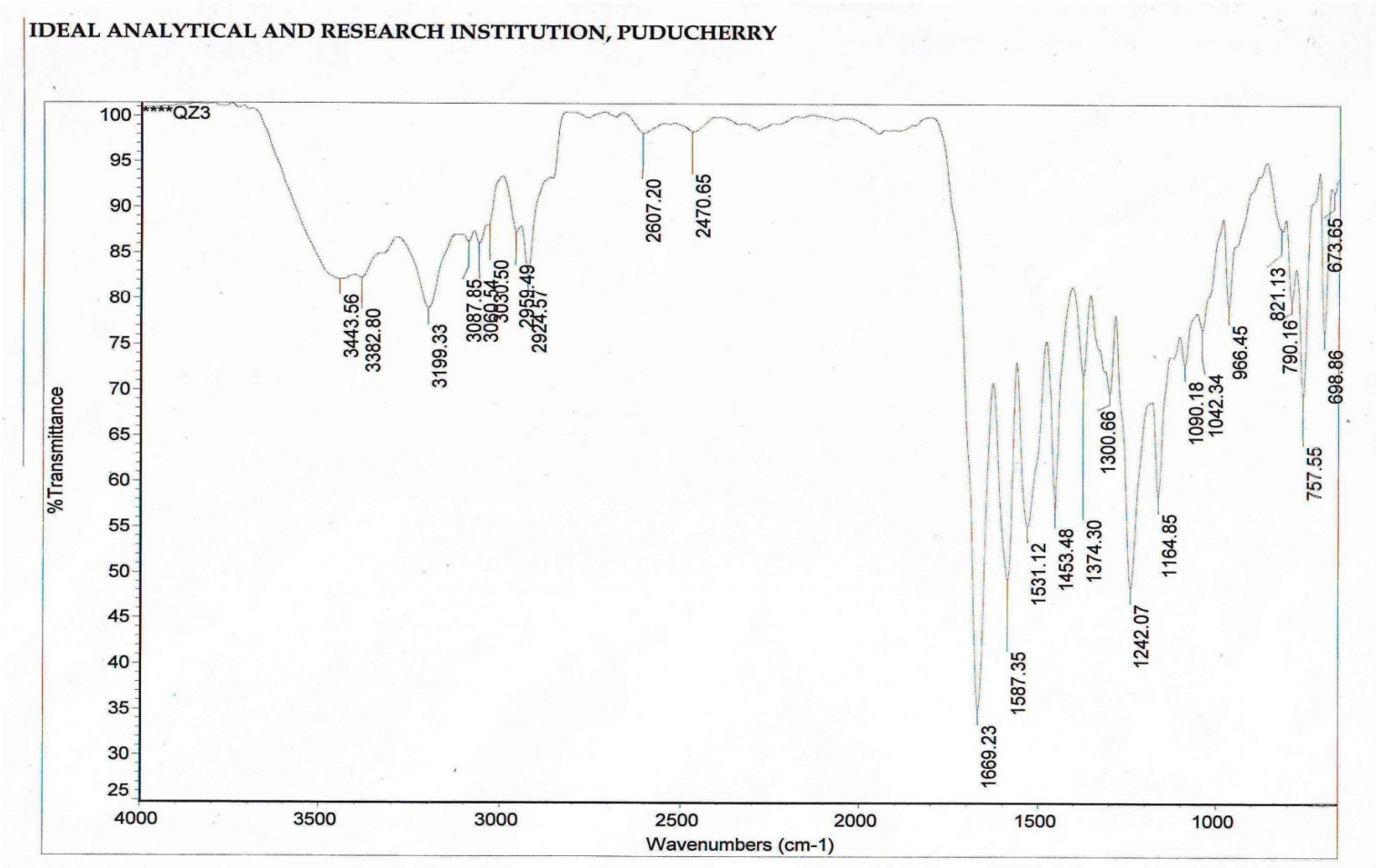
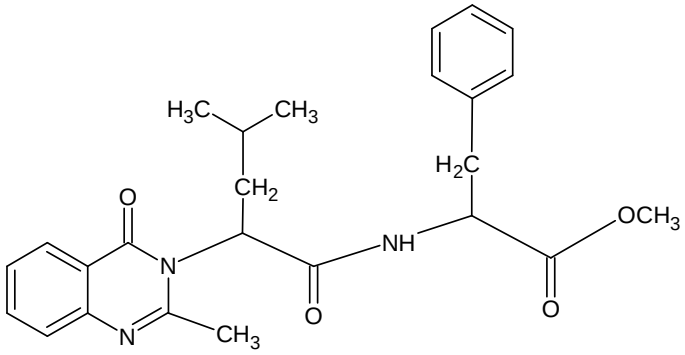


Figure 7: IR Spectra of QZ-3

ii) NMR Spectral Analysis

The proton NMR values were recorded in the BRUKER 500 MHz NMR spectrophotometer.

Interpretation of ^{1}H NMR spectra of MQZ-1



The δ values with reference to the nature of protons are given below**.**

Table-14

S. No	Values in ppm	Nature of protons
1.	8.00	Amide proton in phenylalanine
2.	7.49-7.98	Phenylic proton in quinazolone
3.	7.05-7.16	Phenylic proton in phenylalanine
4.	3.65	Methyl proton in OCH_3
5.	1.94	Methylene proton in leucine
6.	0.90	Gem dimethyl of leucine

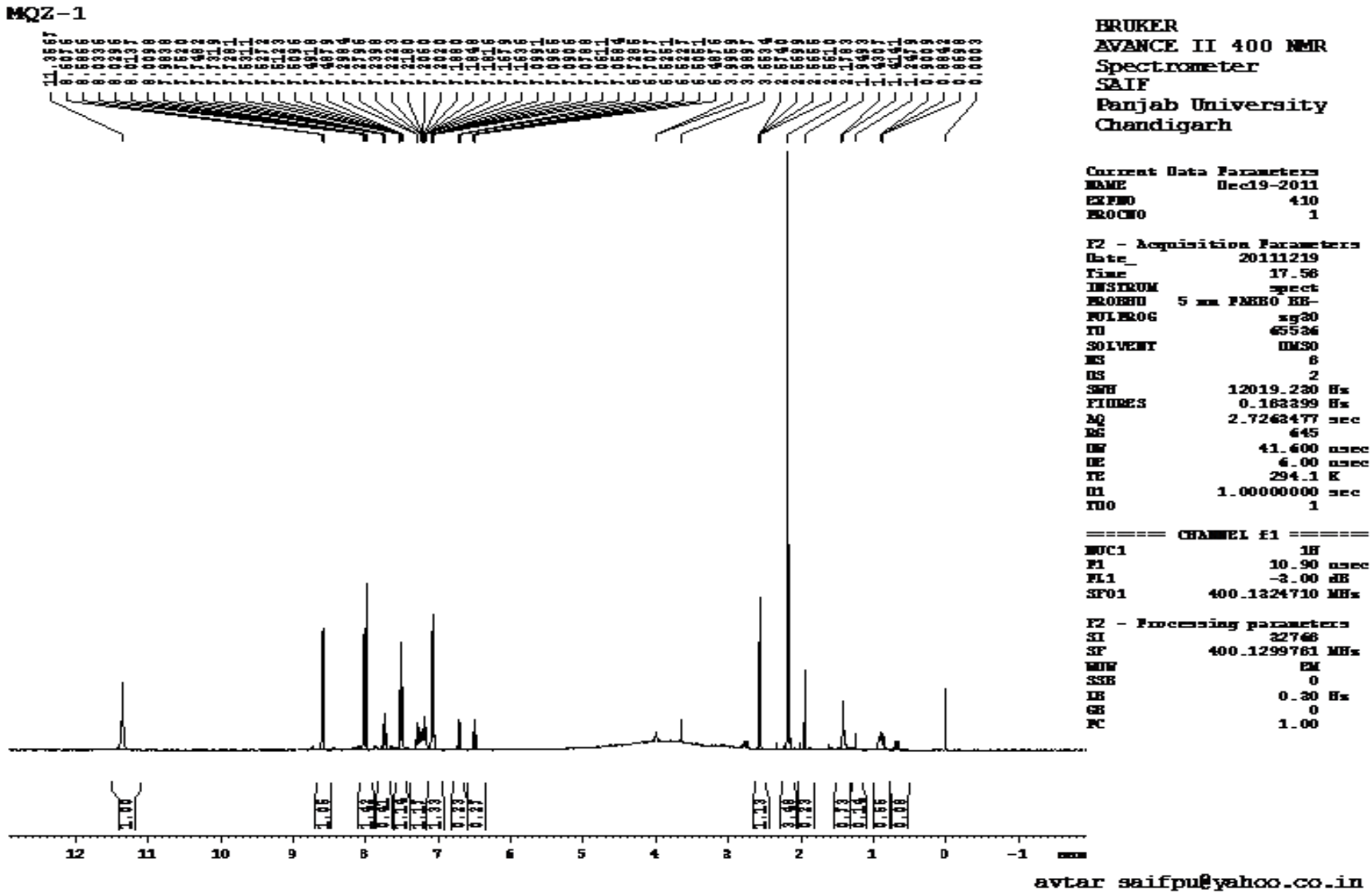
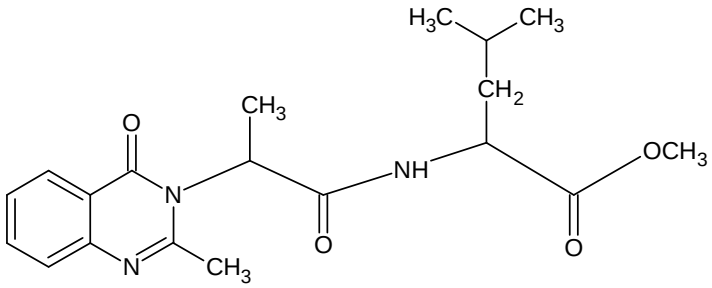


Figure 8: NMR Spectra of MQZ-1

Interpretation of ^{1}H NMR spectra of MQZ-2



The δ values with reference to the nature of protons are given below**.**

Table-15

S. No	Values in ppm	Nature of protons
1.	8.06	Amide proton in phenylalanine
2.	7.51-7.80	Phenylic proton in quinazolone
3.	6.69-7.09	Phenylic proton in phenylalanine
4.	2.58	Methylene proton in phenylalanine
5.	2.16	Methine proton in leucine
6	1.98	Methylene proton in leucine

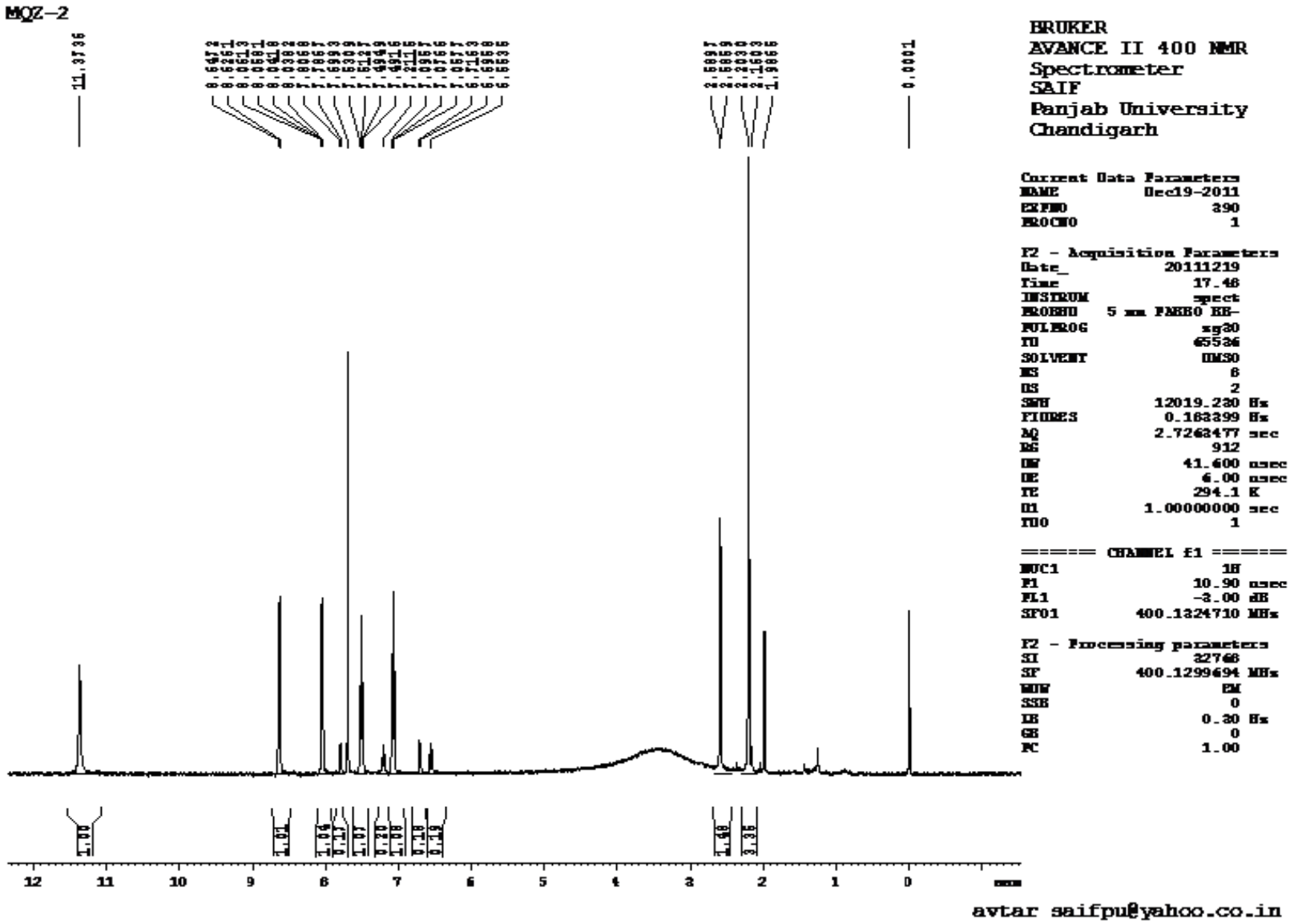
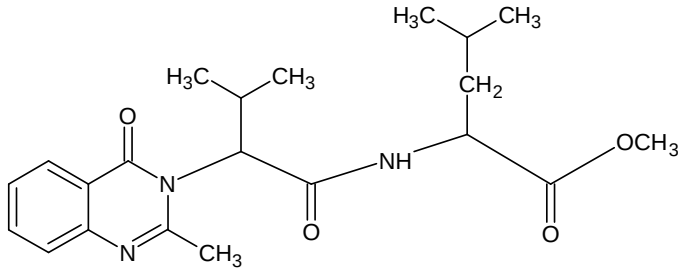


Figure 9: NMR Spectra of MQZ-2

Interpretation of ^{1}H NMR spectra of MQZ-3



The δ values with reference to the nature of protons are given below**.**

Table-16

S. No	Values in ppm	Nature of protons
1.	8.01	Amide proton in leucine
2.	7.49-7.91	Phenylic proton in quinazolone
3.	3.46	Methyl proton in OCH_3
4.	2.18	Methine proton in leucine
5.	2.57	Methine proton in valine
6.	1.95	Methylene proton in leucine

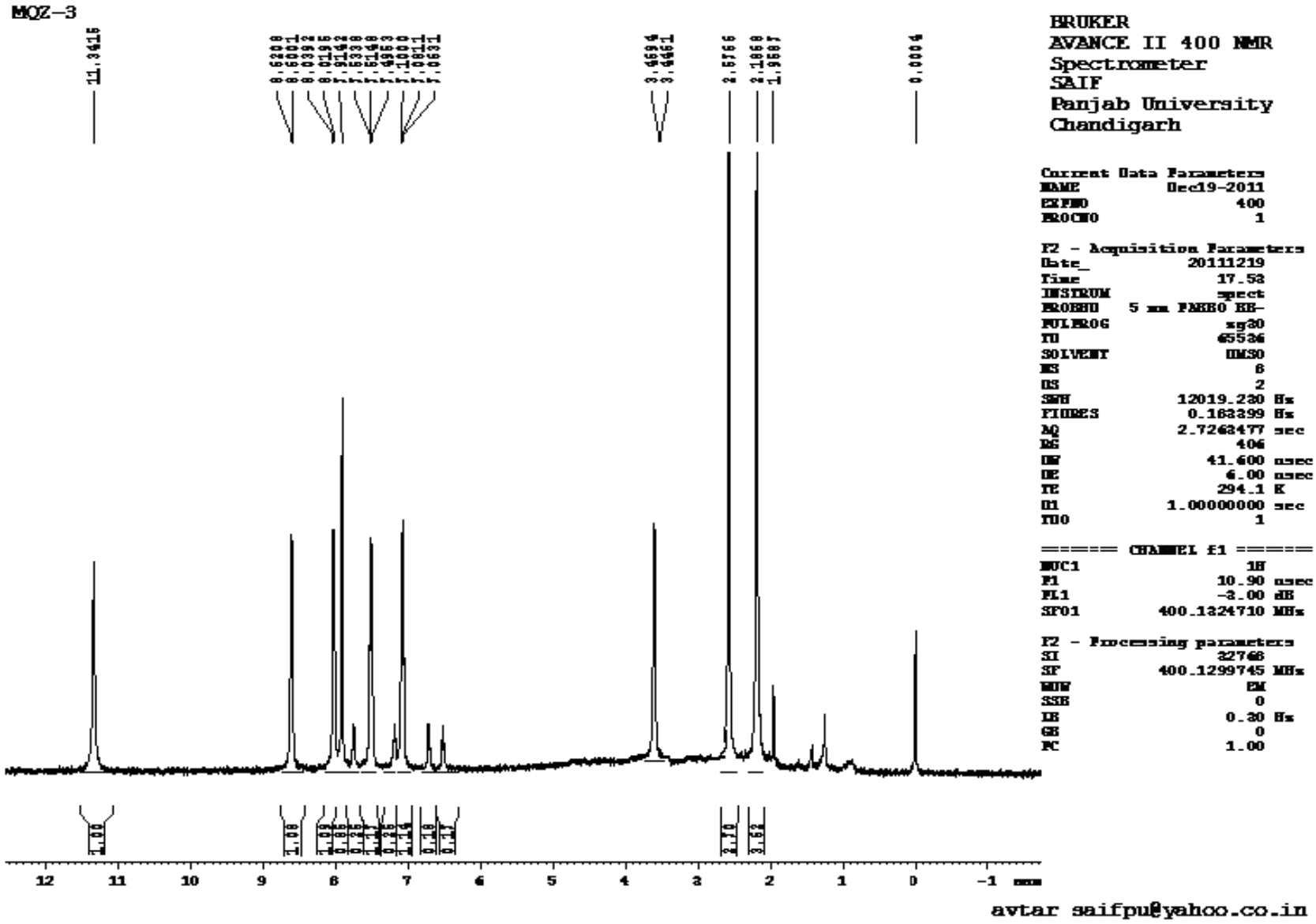
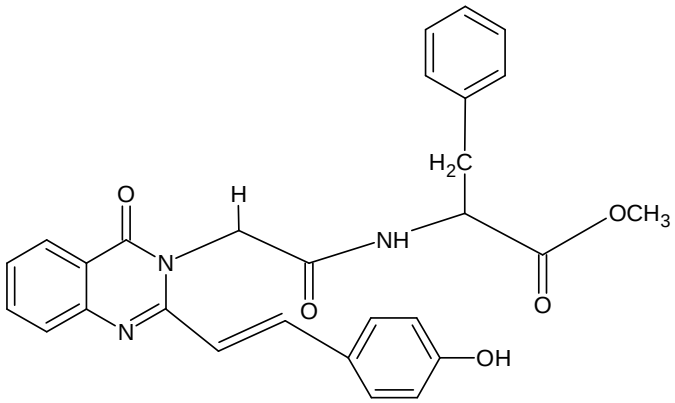


Figure 10: NMR Spectra of MQZ-3

Interpretation of ^{1}H NMR spectra of QZ-1



The δ values with reference to the nature of protons are given below**.**

Table-17

S. No	Values in ppm	Nature of protons
1.	7.83	Amide proton in phenylalanine
2.	7.56	Phenylic proton in quinazolone
3.	6.71	Olefinic CH proton of styryl group
4.	3.62	Methlyene proton in glycine
5.	3.52	Methyl proton in OCH_3
6.	2.58	Methlyene proton in phenylalanine

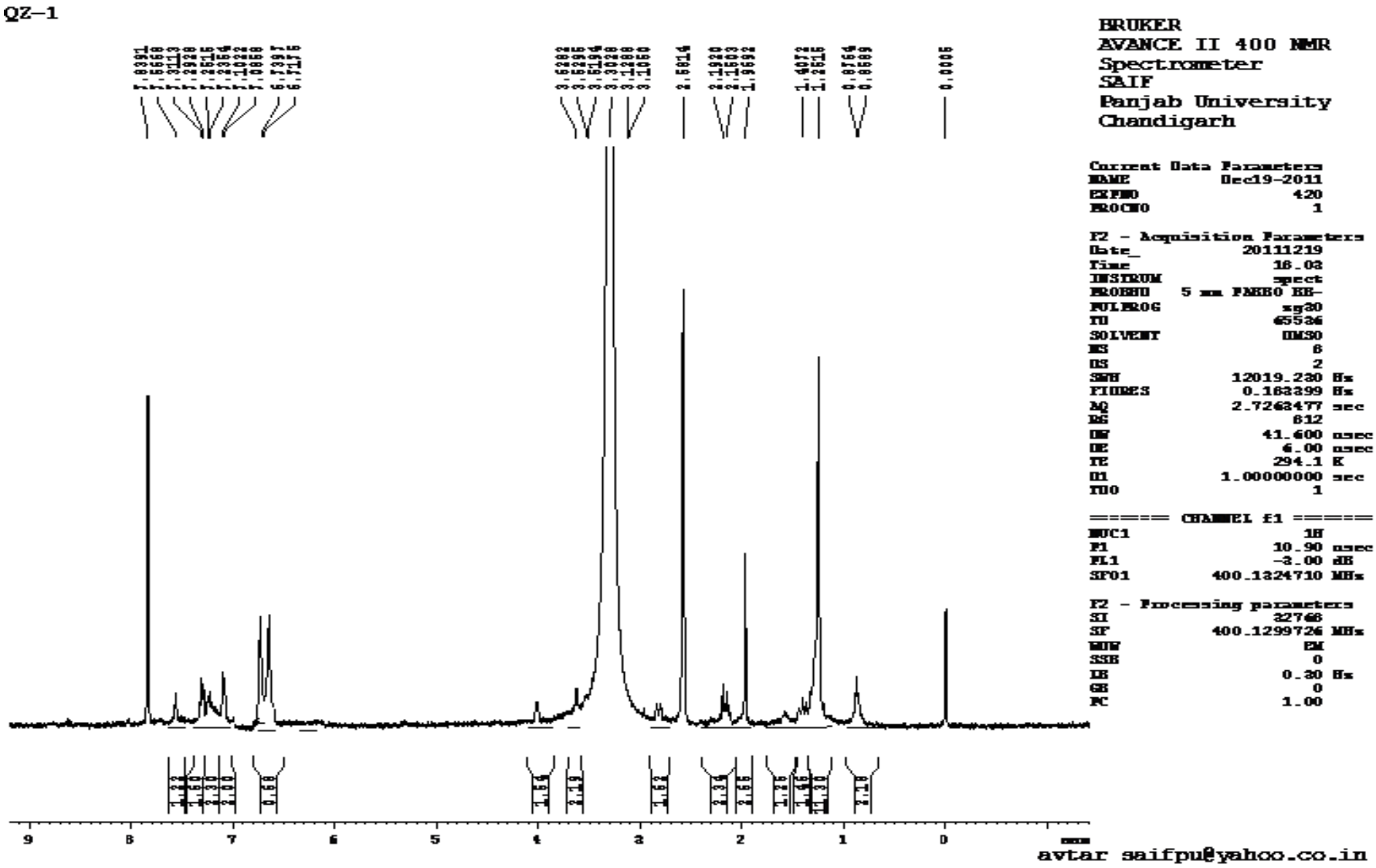
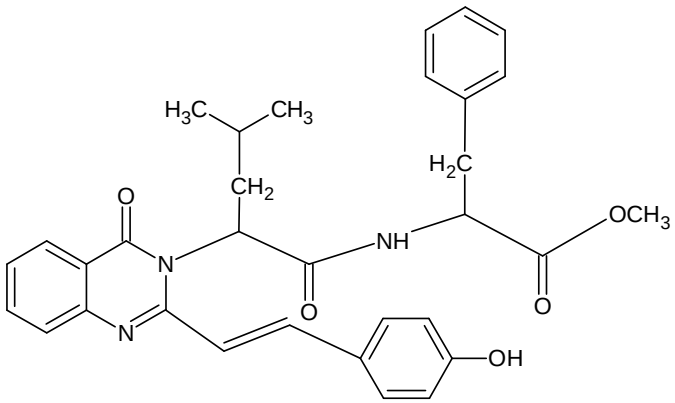


Figure 11: NMR Spectra of QZ-1

Interpretation of ^{1}H NMR spectra of QZ- 2



The δ values with reference to the nature of protons are given below**.**

Table-18

S. No	Values in ppm	Nature of protons
1.	8.02	Amide proton in phenylalanine
2.	7.49-7.86	Phenylic proton in quinazolone
3.	7.05-7.09	Phenylic proton in phenylalanine
4.	6.91	Olefinic CH proton of styryl group
5.	2.19	Methine proton in leucine
6.	1.15	Gem dimethyl of leucine

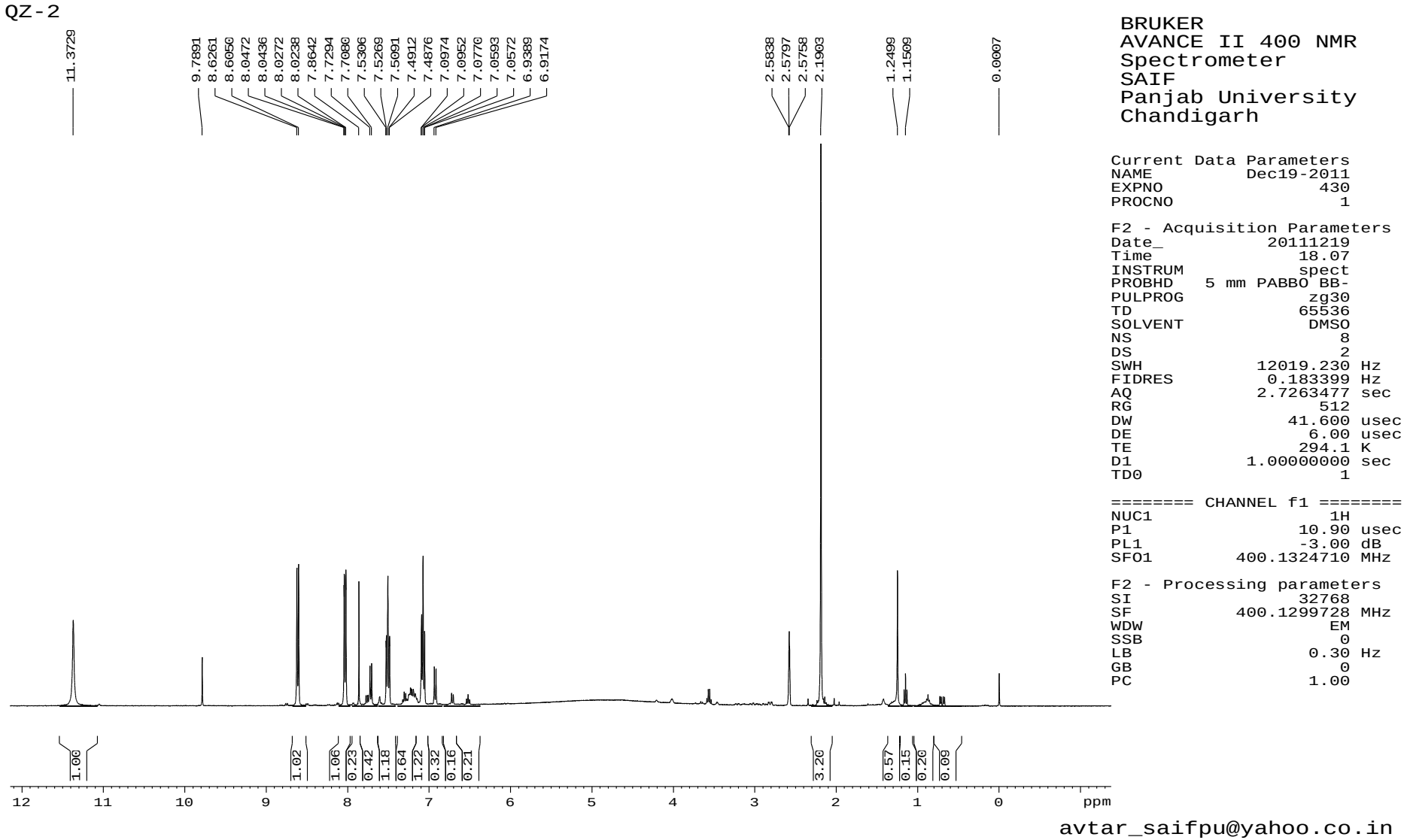
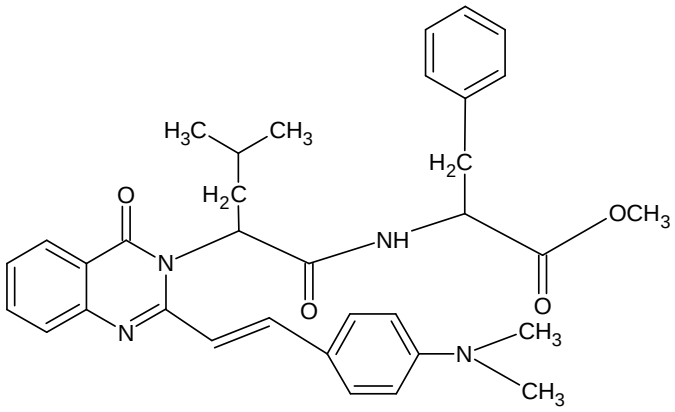


Figure 12: NMR Spectra of QZ-2

Interpretation of ^{1}H NMR spectra of QZ- 3



The δ values with reference to the nature of protons are given below**.**

Table-19

S. No	Values in ppm	Nature of protons
1.	8.00	Amide proton in phenylalanine
2.	7.52-7.92	Phenylic proton in quinazolone
3.	6.71	Olefinic CH proton of styryl group
4.	4.19	Methine proton of leucine
5.	2.83	CH_3 proton of $N(CH_3)_2$
6.	2.18	Methylene proton in leucine

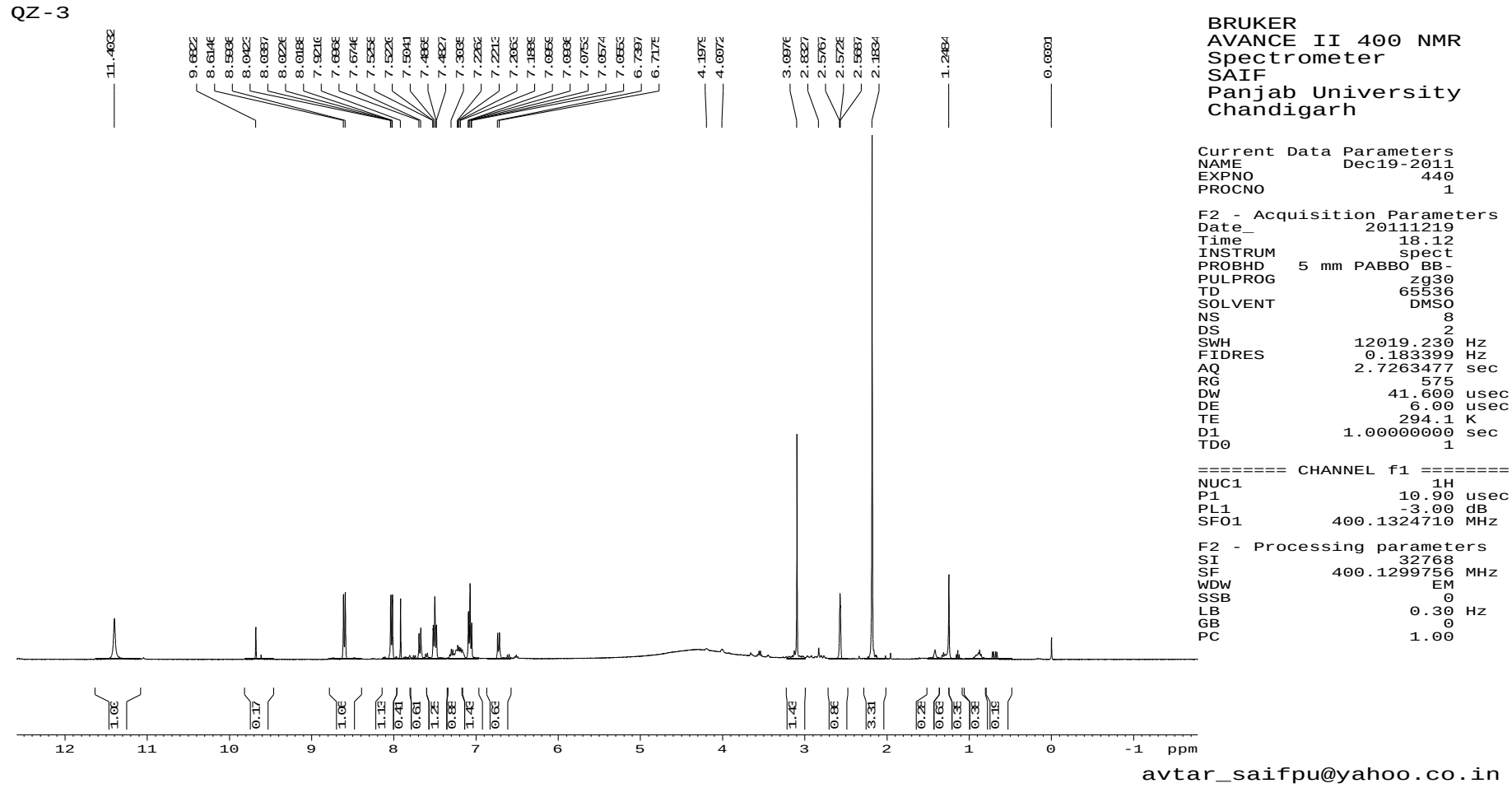
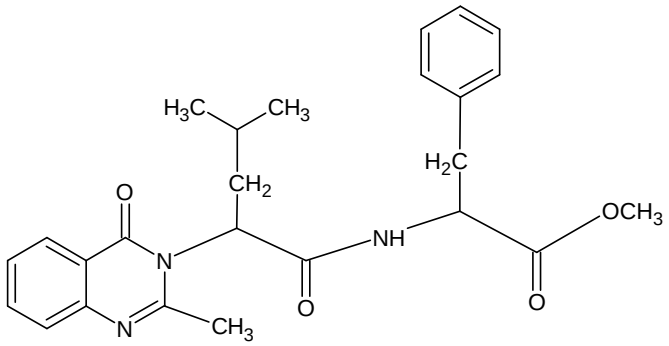


Figure 13: NMR Spectra of QZ-3

iii) Mass Spectral Analysis

Mass spectrum of the sample was recorded in JOEL GC mate by electron impact method as ionization mode.

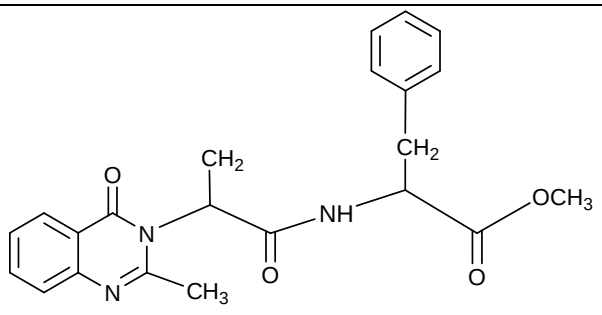
Interpretation of Mass spectra of MQZ-1

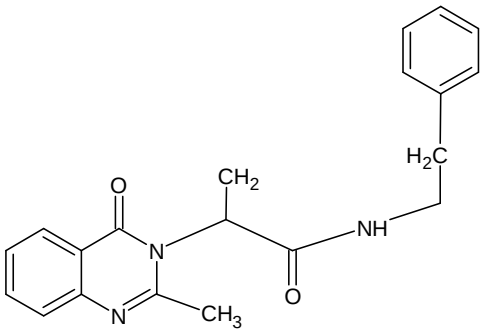
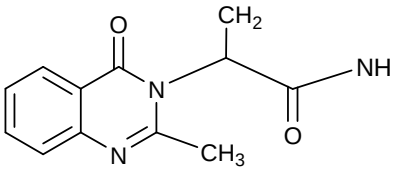
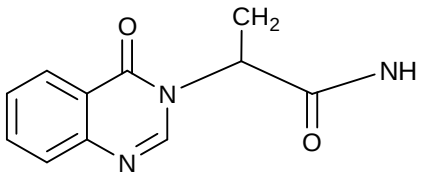
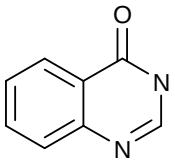


Molecular Weight: 435.51 Molecular Ion Peak: 435.12

The possible fragments of the molecule with the relevant to its m/z values are:

Table -20

S. No	m/z	Fragments
1.	392.16	

2.	333.14	
3.	229.08	
4.	215.06	
5.	145.05	

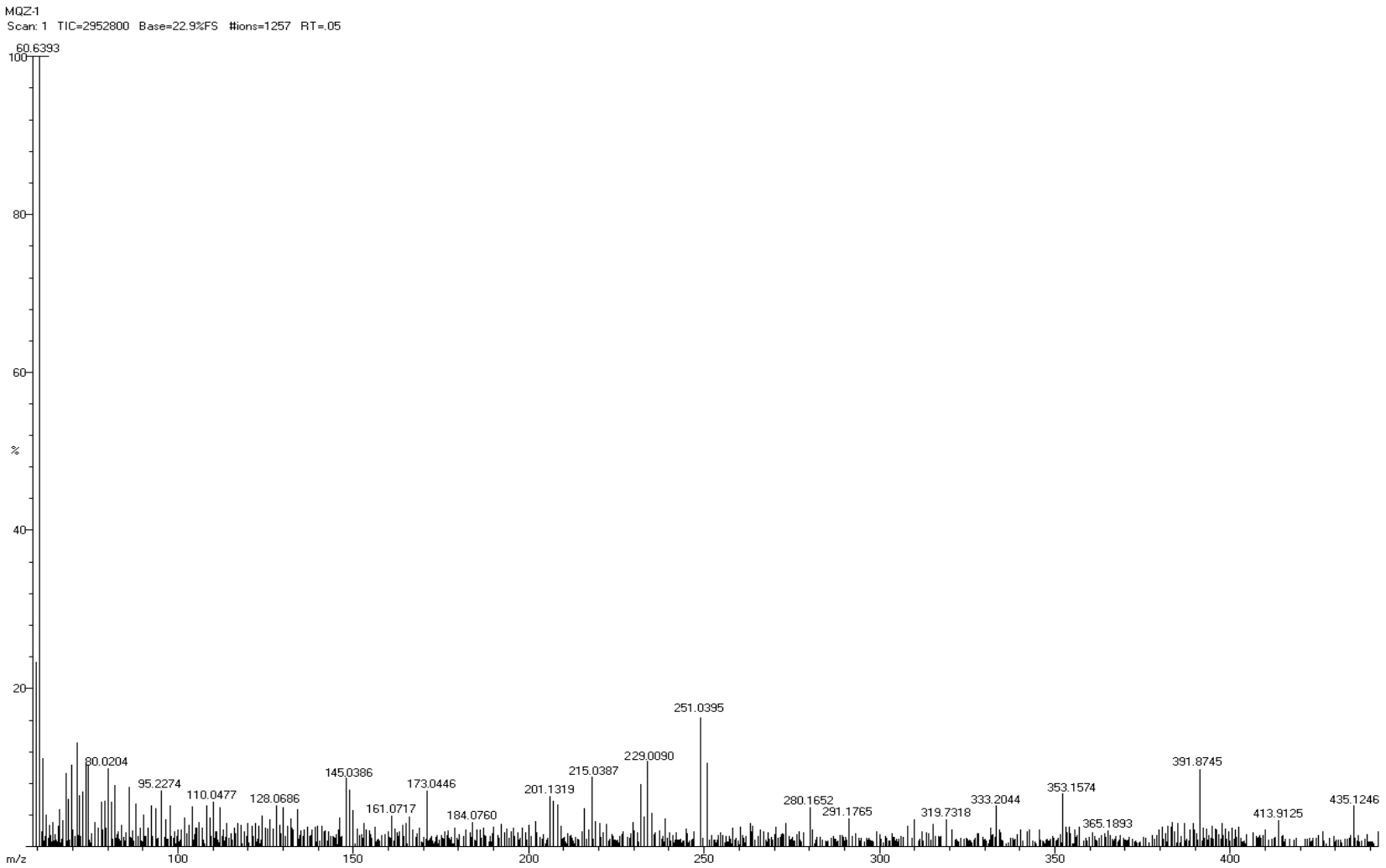
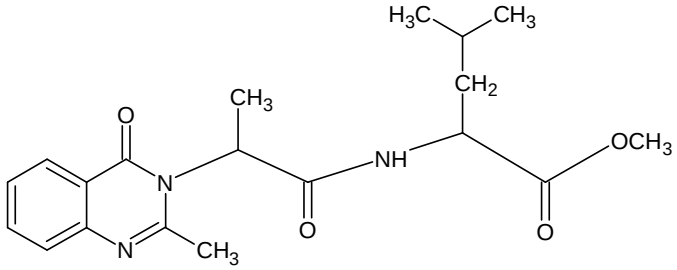


Figure 14: Mass Spectra of MQZ-1

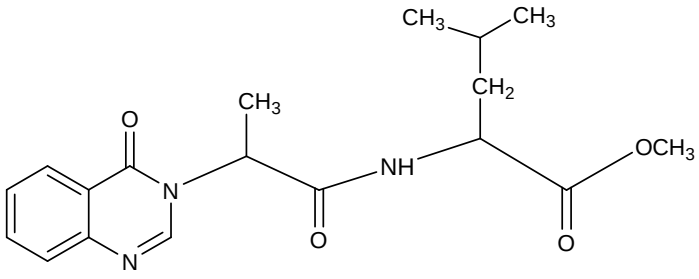
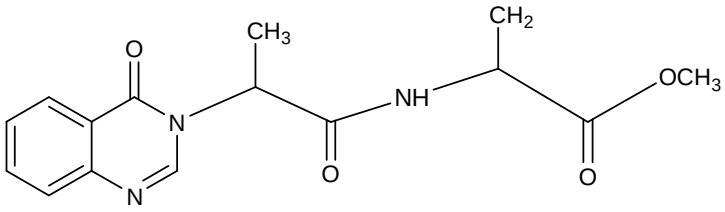
Interpretation of Mass spectra of MQZ-2

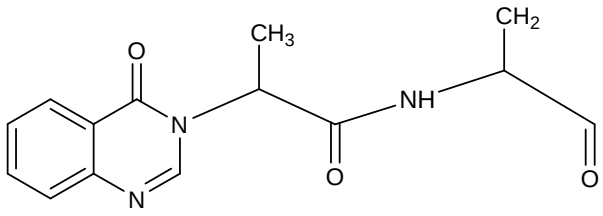
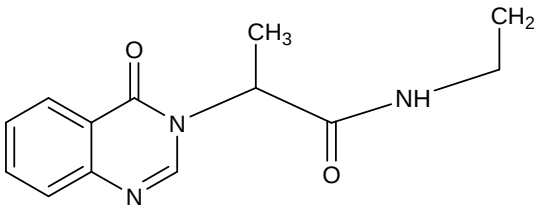
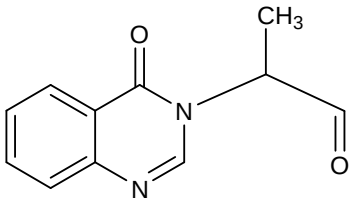


Molecular Weight: 359.41 Molecular Ion Peak: 359.00

The possible fragments of the molecule with the relevant to its m/z values are:

Table -21

S. No	m/z	Fragments
1.	345.16	
2.	302.11	

3.	272.09	
4.	244.12	
5.	202.21	

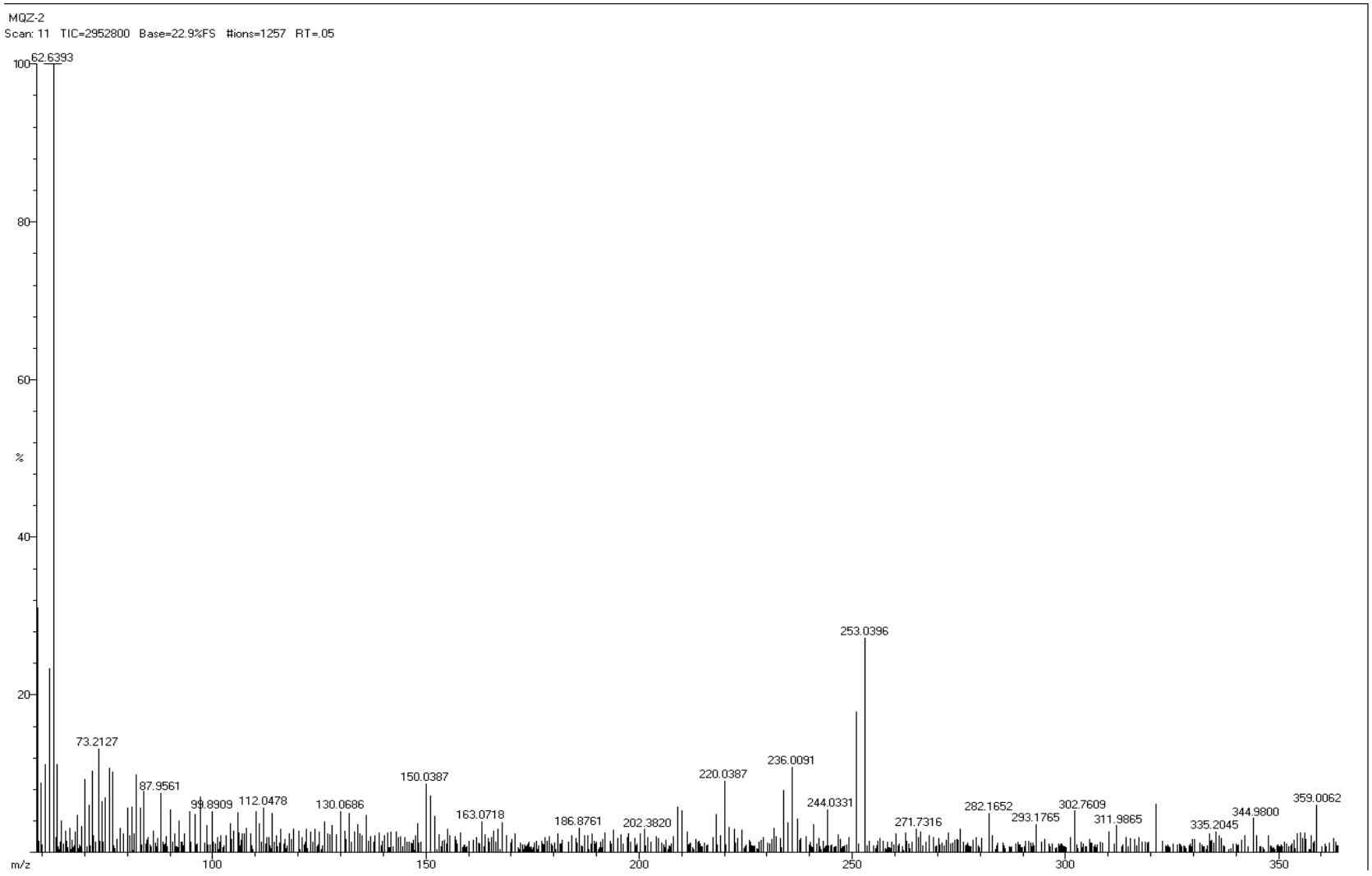
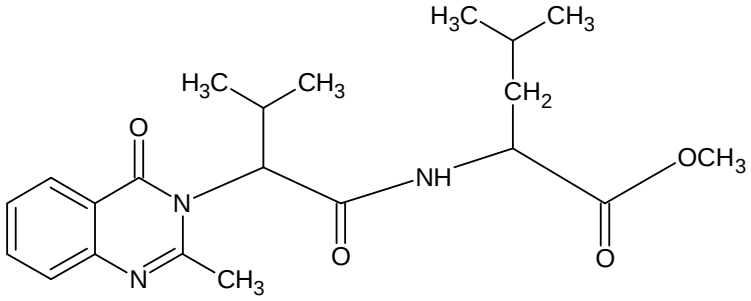


Figure 15: Mass Spectra of MQZ-2

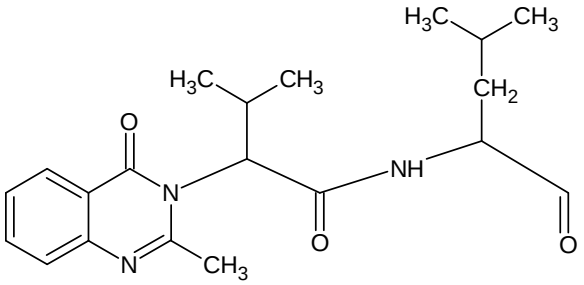
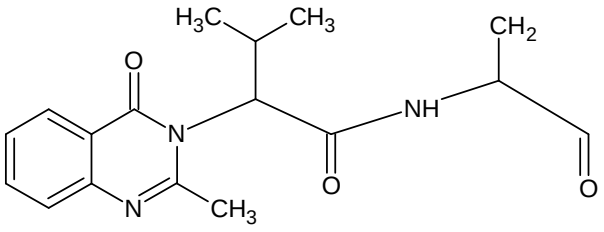
Interpretation of Mass spectra of MQZ-3

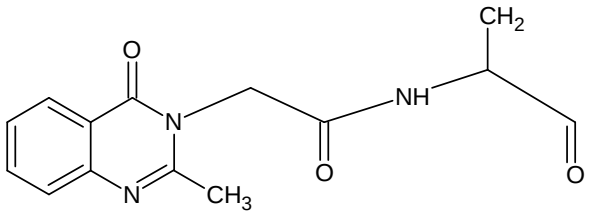
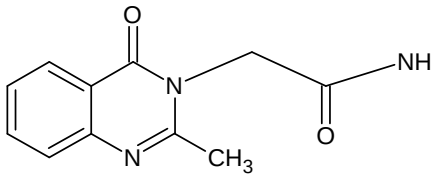
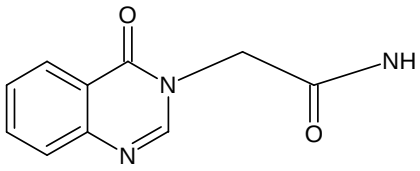


Molecular Weight: 387.47 Molecular Ion Peak: 387.83

The possible fragments of the molecule with the relevant to its m/z values are:

Table-22

S. No	m/z	Fragments
1.	357.44	
2.	314.35	

3.	272.27	
4.	216.07	
5.	202.18	

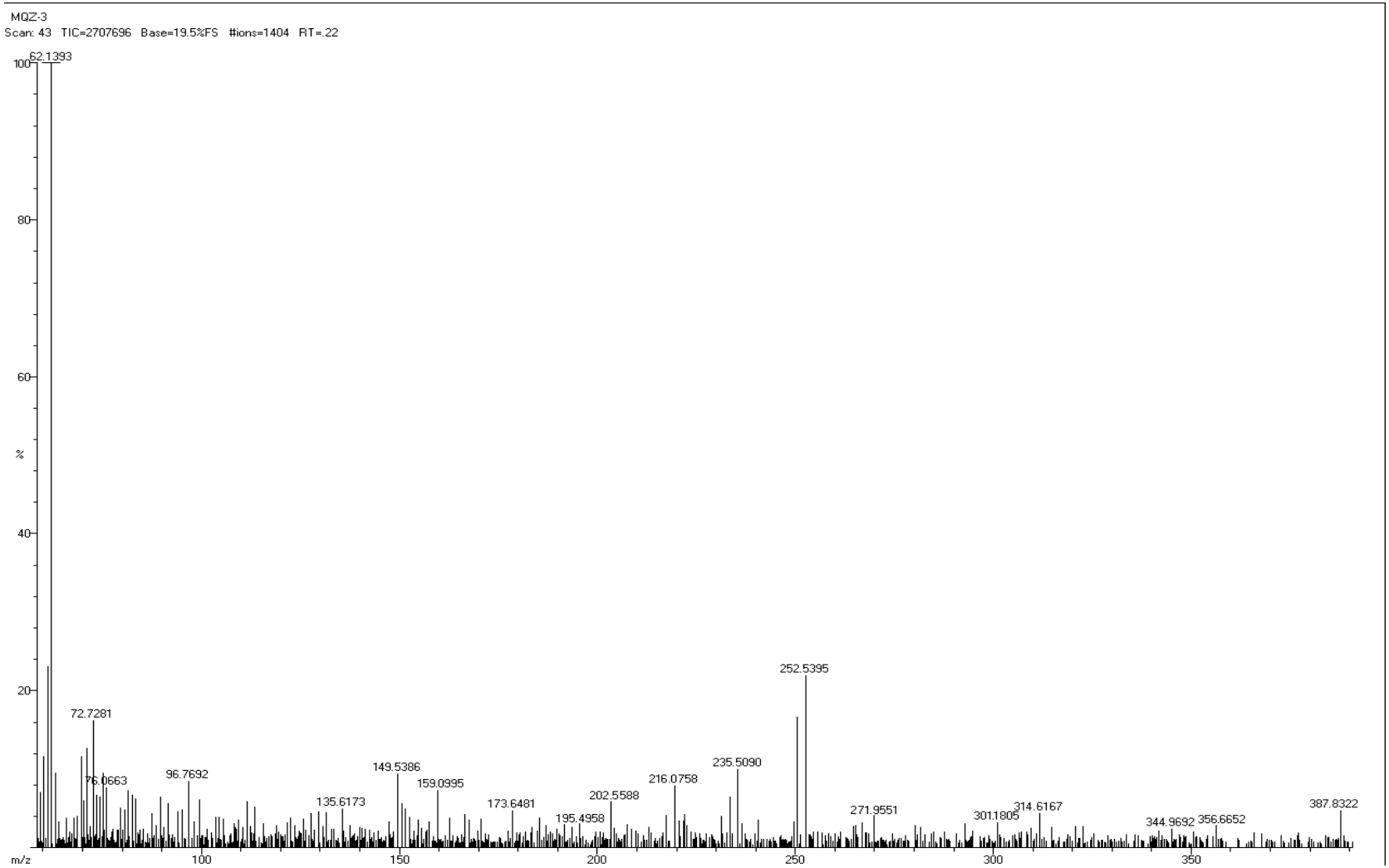
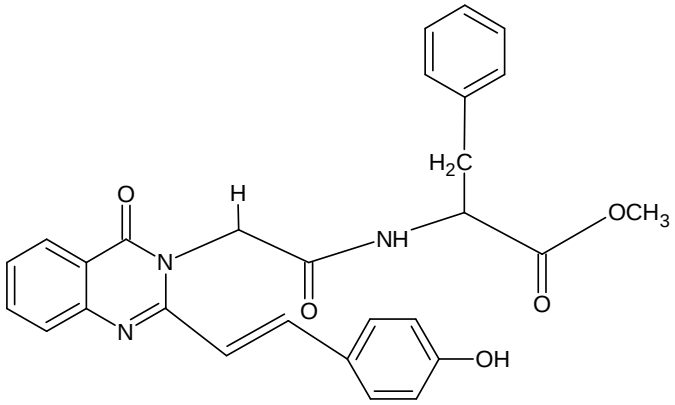


Figure 16: Mass Spectra of MQZ-3

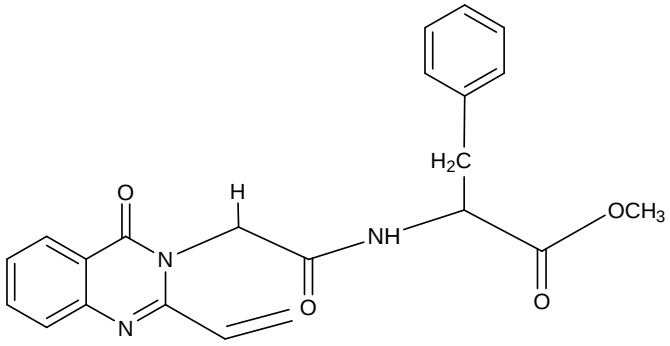
Interpretation of Mass spectra of QZ-1

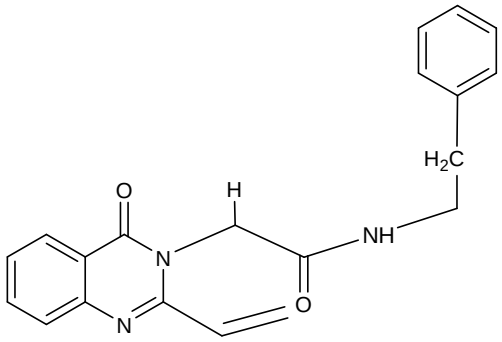
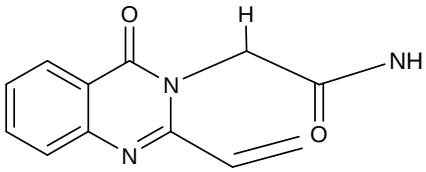
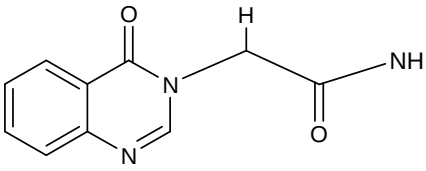
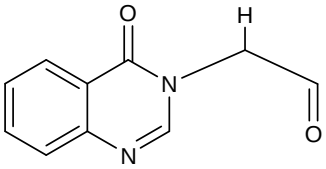


Molecular Weight: 483.52 Molecular Ion Peak: 483.77

The possible fragments of the molecule with the relevant to its m/z values are:

Table-23

S. No	m/z	Fragments
1.	390.15	

2.	333.15	
3.	228.23	
4.	202.19	
5.	188.18	

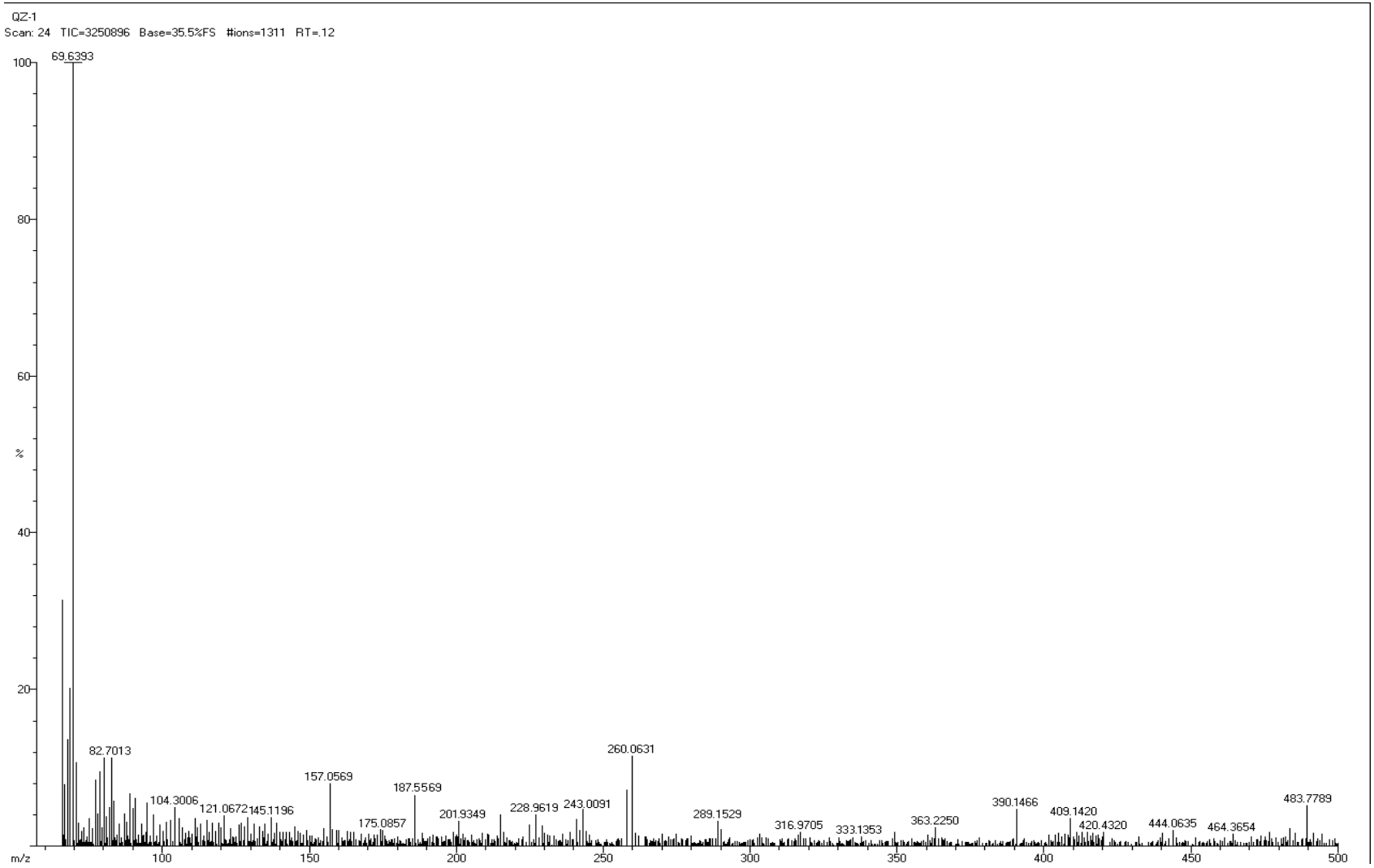
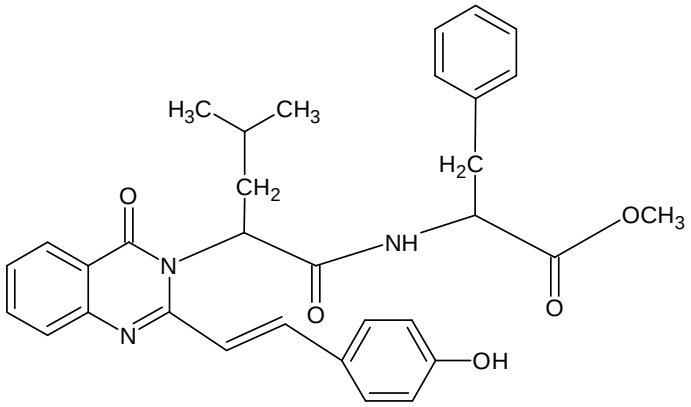


Figure 17: Mass Spectra of QZ-1

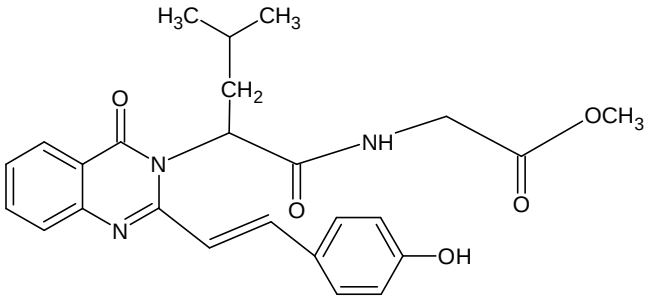
Interpretation of Mass spectra of QZ-2

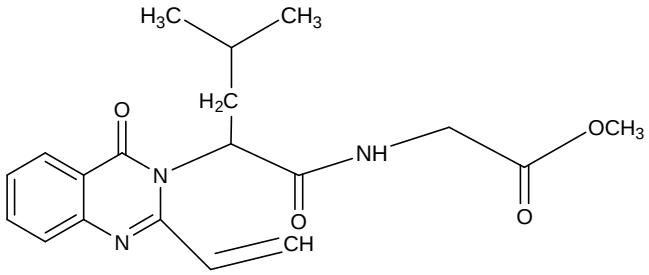
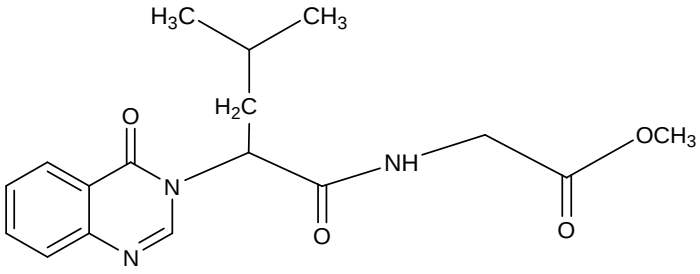
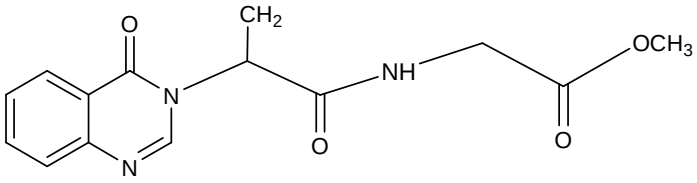
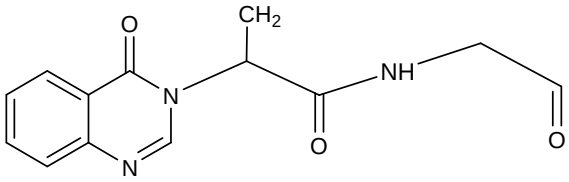


Molecular Weight: 539.62 Molecular Ion Peak: 539.62

The possible fragments of the molecule with the relevant to its m/z values are:

Table-24

S. No	m/z	Fragments
1.	449.20	

2.	357.40	
3.	331.15	
4.	288.28	
5.	258.25	

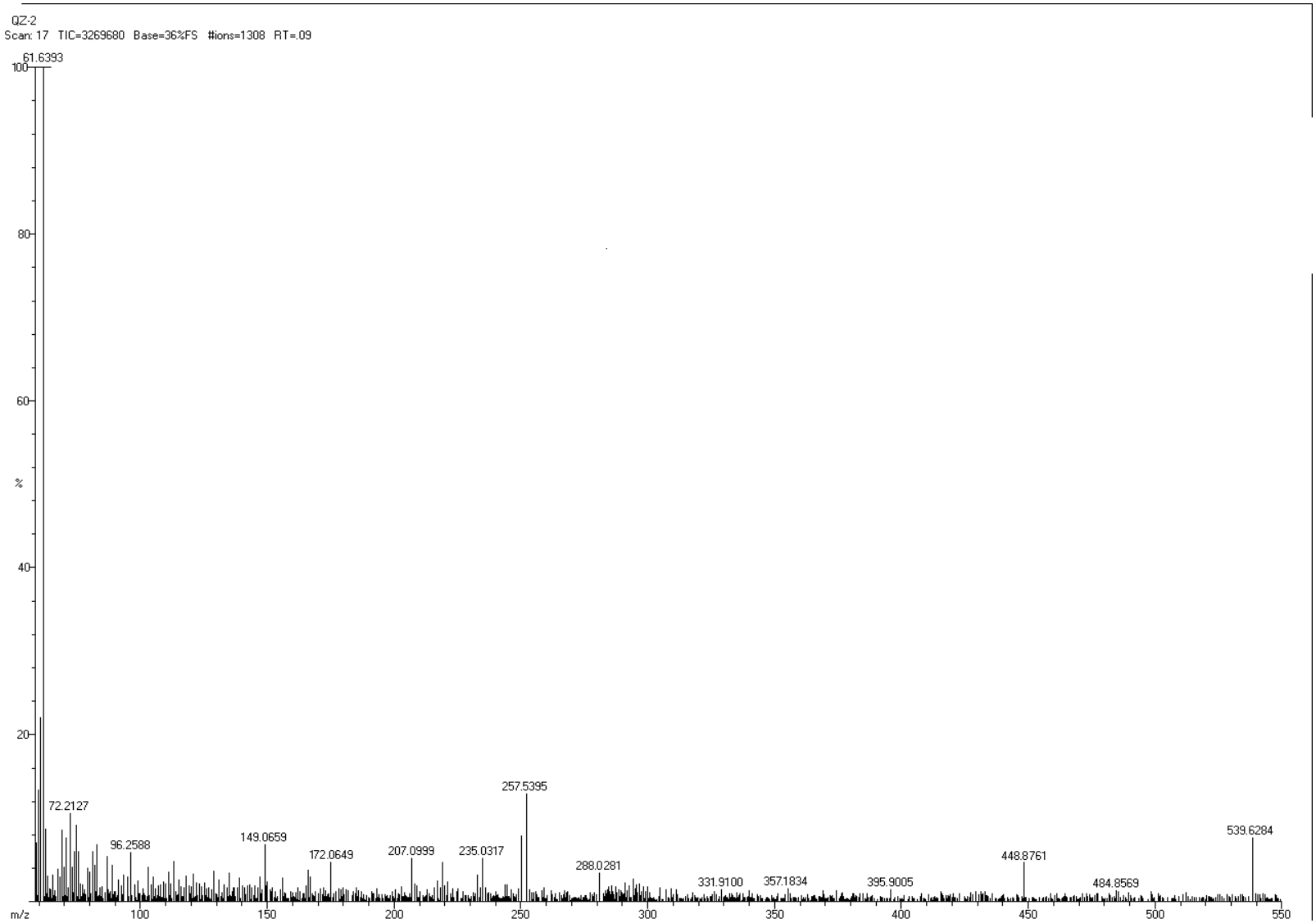
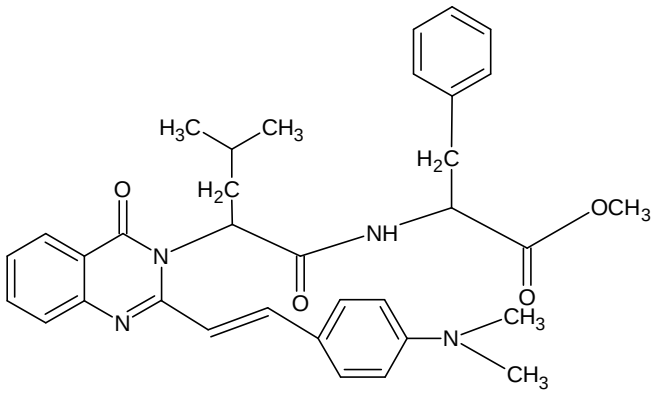


Figure 18: Mass Spectra of QZ-2

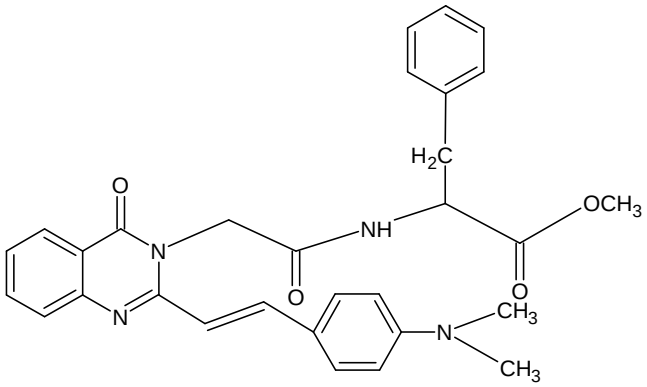
Interpretation of Mass spectra of QZ-3

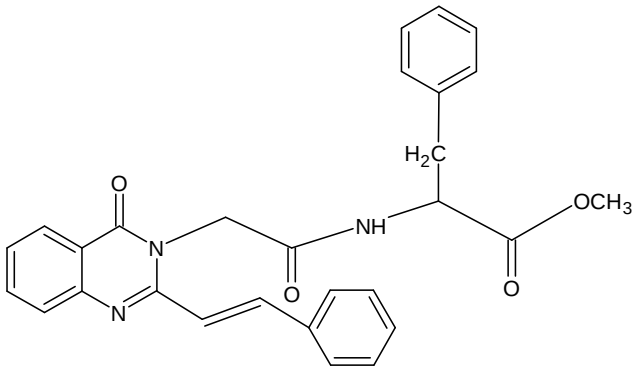
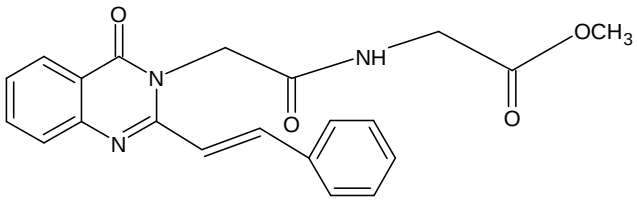
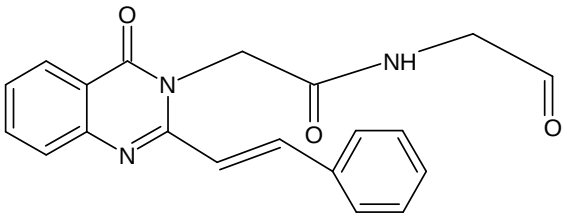
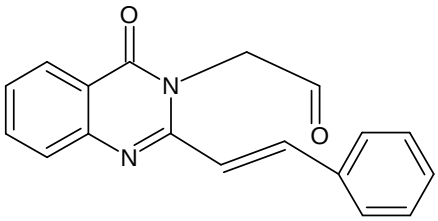


Molecular Weight: 566.69 Molecular Ion Peak: 566.52

The possible fragments of the molecule with the relevant to its m/z values are:

Table-25

S. No	m/z	Fragments
1.	510.58	

2.	467.51	
3.	377.39	
4.	347.36	
5.	290.31	

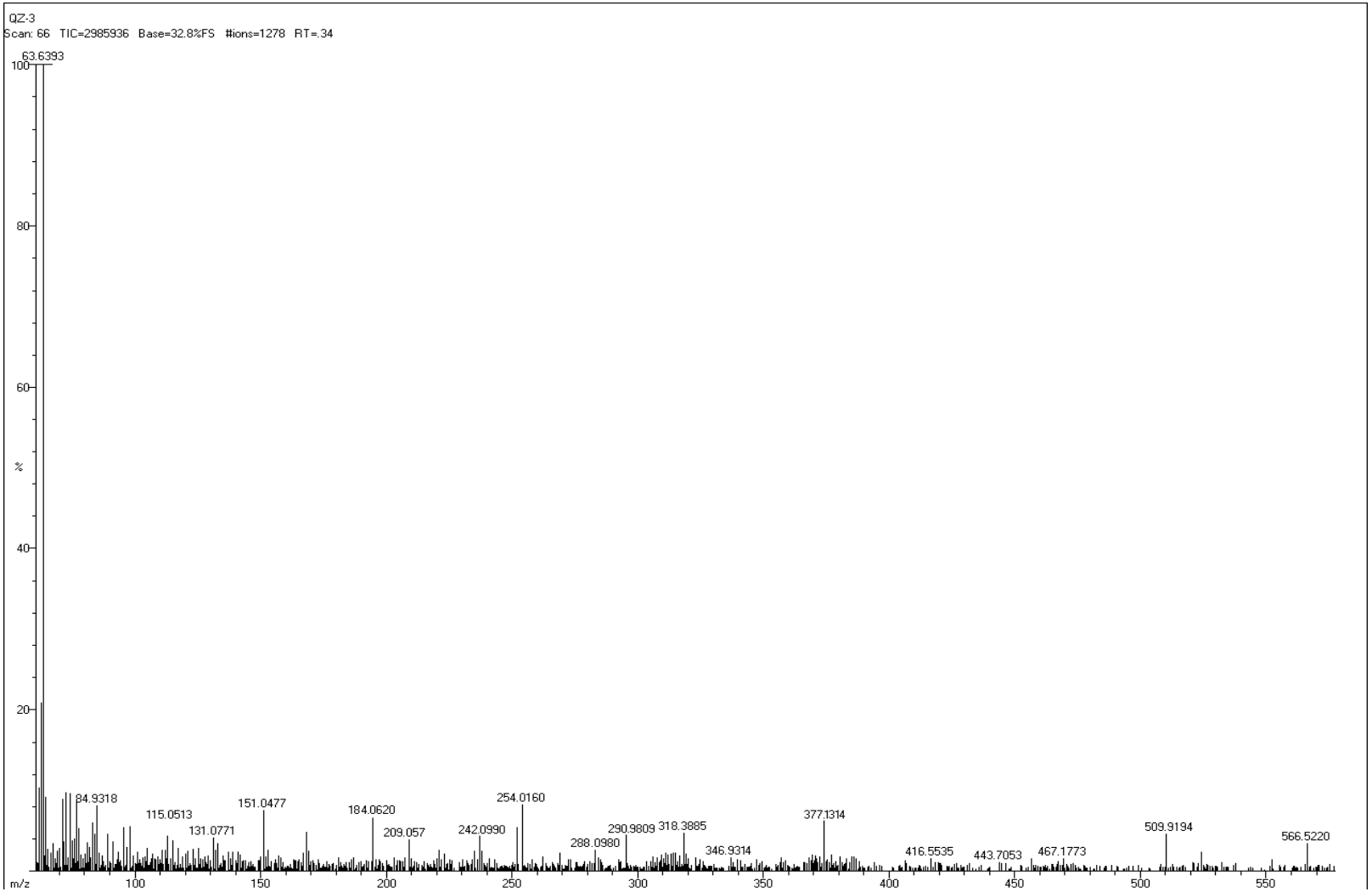


Figure 19: Mass Spectra of QZ-3

C. RESULTS OF ANALGESIC ACTIVITY

The analgesic activity of the synthesized compounds was evaluated by Eddy's hot plate method using pentazocine as standard.

Table-26

Compound	Paw licking or jumping response (in seconds)				
	0 min	**30 mins**	**60 mins**	**90 mins**	**180 mins**
Control	7.02 ± 0.58	8.06 ± 0.58	7.08 ± 0.58	7.01 ± 0.58	6.67 ± 0.67
Standard	10.04 ± 1.15	10.21 ± 0.58	11.03 ± 0.58**	10.67 ± 0.33***	9.52 ± 0.88***
QZ-1	8.60 ± 0.50	7.66 ± 0.32	9.33 ± 0.47*	9.6 ± 0.26***	8.02 ± 0.66*
QZ-2	8.32 ± 0.88	7.55 ± 0.42	8.38 ± 0.88**	8.66 ± 0.71**	7.66 ± 0.32*

*P<0.05, **P<0.01. ***P<0.001 compare to control response. One-way ANOVA followed by Bonferroni test.

D. SCREENING OF ANTIMICROBIAL ACTIVITY

i) Results of Antibacterial activity

The antibacterial activity of synthesized compounds were screened against each gram positive (*Micrococlus luteus*) and gram negative organism (*Klebsiella pneumoniae)* by disc diffusion method using ciprofloxacin as standard drug.

Table-27

Compound	Zone of Inhibition (in mm)					
	Micrococlus luteus **(µg/ml)**			***Klebsiella pneumoniae*** **(µg/ml)**		
	50	**100**	**150**	**50**	**100**	**150**
QZ-1	11	19	24	16	20	23
QZ-2	13	20	25	14	20	24
QZ-3	15	20	27	15	19	23
QZ-4	12	14	20	13	21	21
QZ-5	10	13	19	12	17	20
QZ-6	11	14	18	13	15	19
Ciprofloxacin (10µg/ml)	39			38		

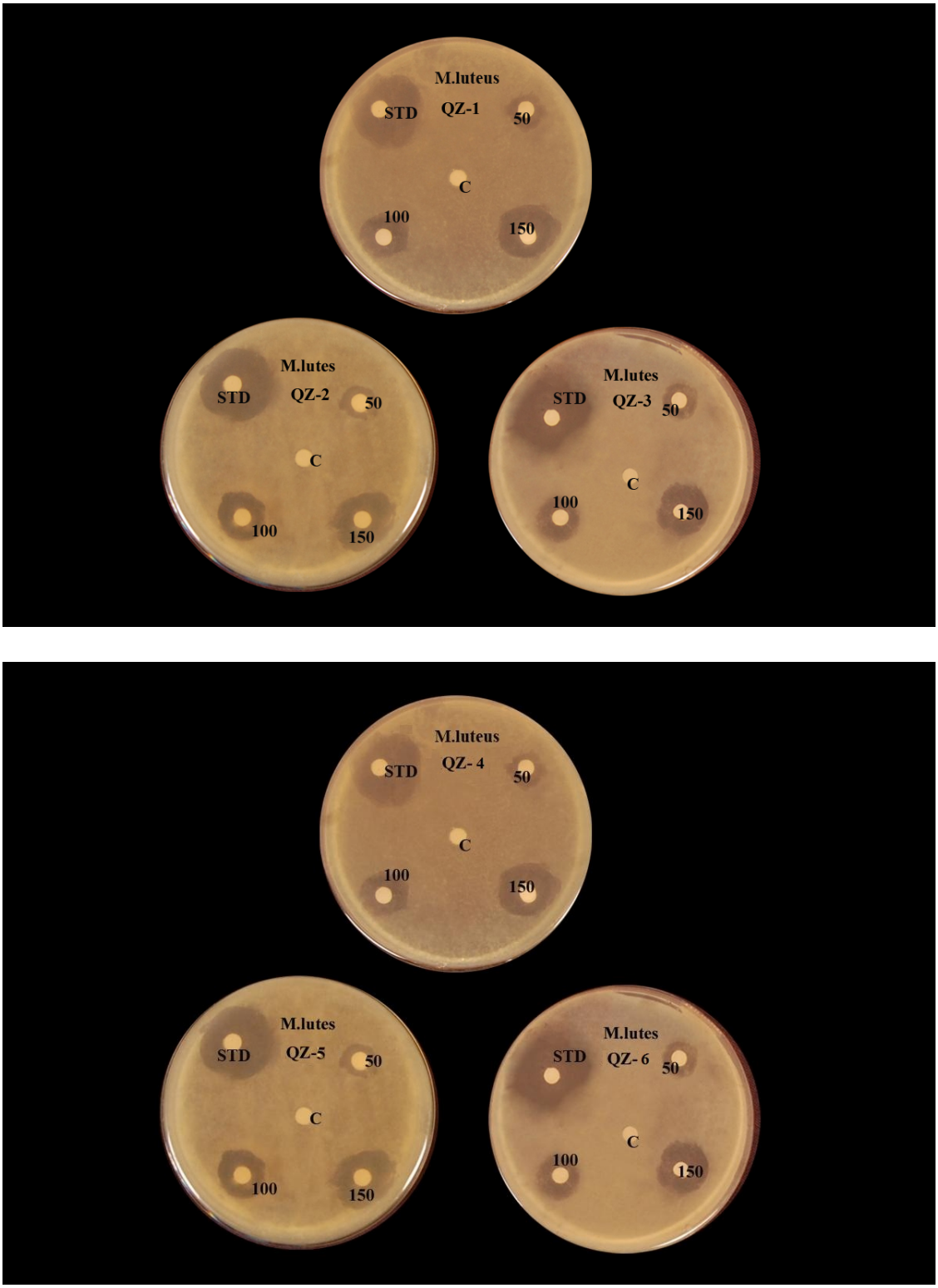


Fig: 20 Antibacterial activity of synthesized compounds against *Micrococlus luteus*

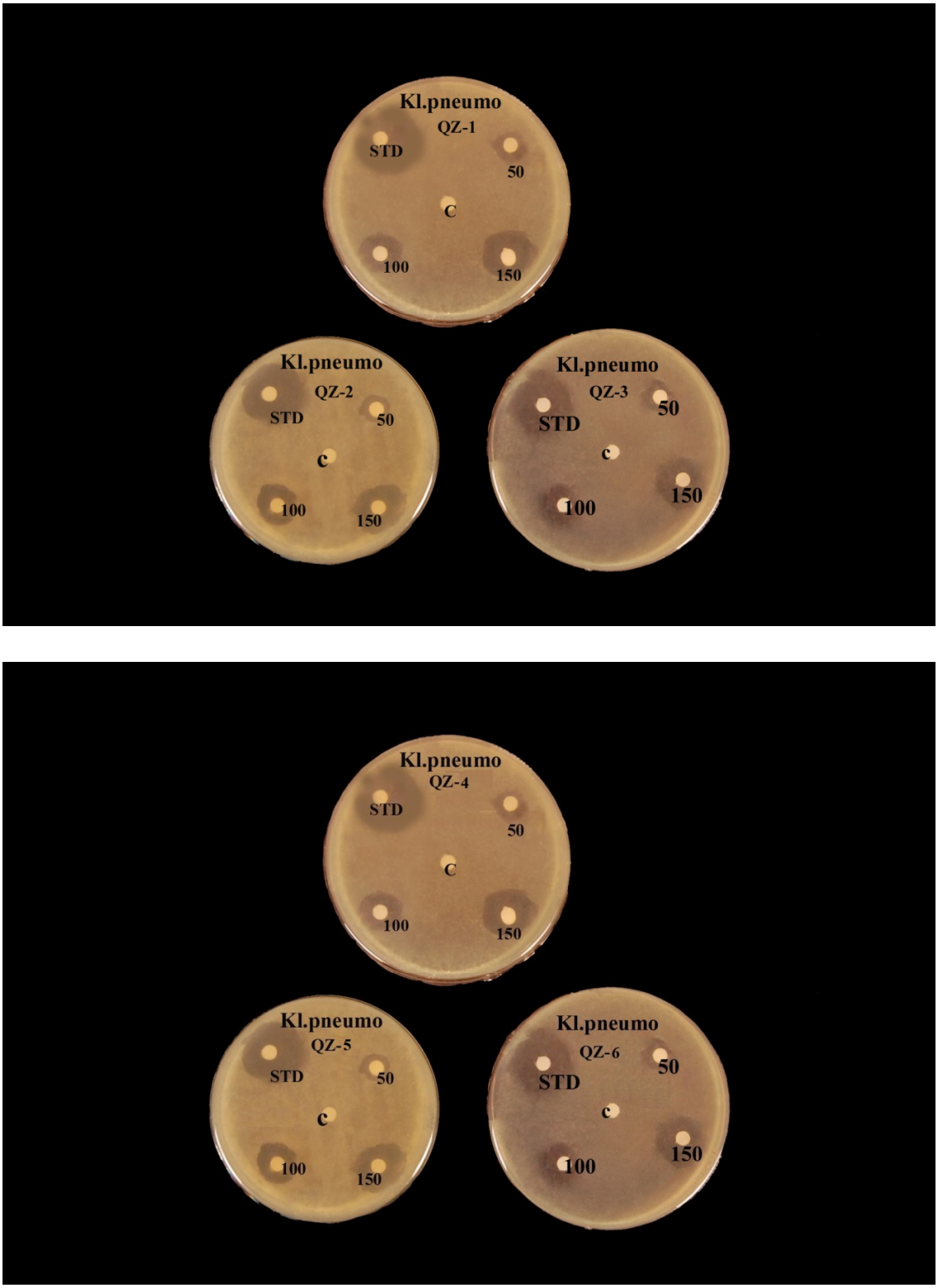


Fig: 21 Antibacterial activity of synthesized compounds against *Klebsiella pneumoniae*

ii) Results of Antifungal activity

The antifungal activity of synthesized compounds were screened against *Candida albicans* by disc diffusion method using ketaconazole as standard drug

Table-28

Compound	**Zone of Inhibition (in mm)**		
	Candida albicans **(µg/ml)**		
	50	**100**	**150**
QZ-1	20	22	26
QZ-2	19	22	25
QZ-3	18	21	24
QZ-4	17	20	21
QZ-5	18	19	21
QZ-6	16	19	20
Ketaconazole (10µg/ml)	38		

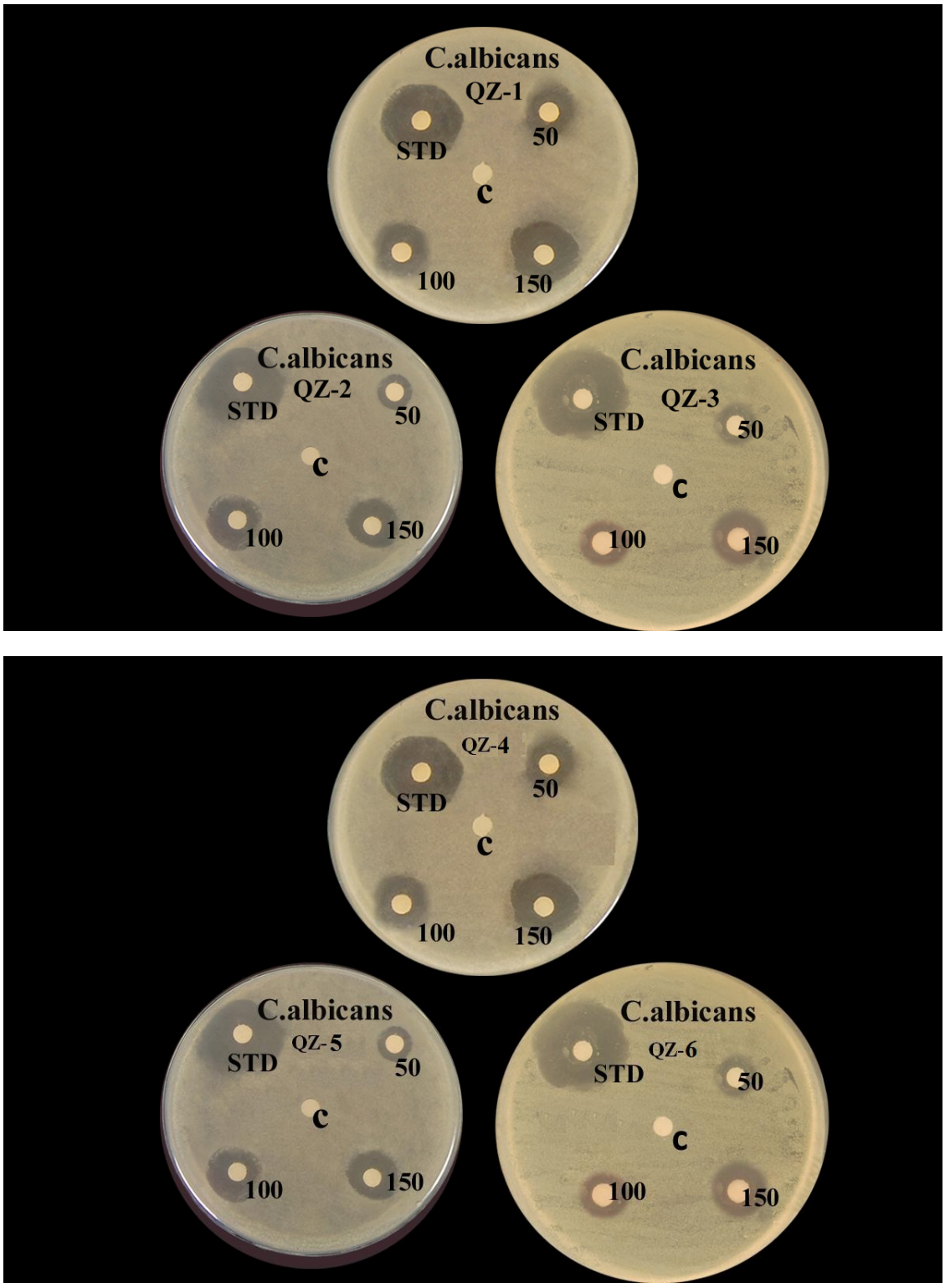


Fig: 22 Antifungal activity of synthesized compounds against *Candida albicans*

DISCUSSION

Quinazolone derivatives were synthesized and the melting point was determined and the completion of reaction was checked by thin layer chromatography using silica gel as stationary phase and hexane:ethyl acetate (8:2) as mobile phase. The spot in the TLC plate was detected by iodine vapours. The chemical structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral data. And the synthesized compounds were screened for analgesic and antimicrobial activities.

Analgesic activity

The synthesized compounds were screened for analgesic activity against thermal stimuli by Eddy's hot plate method in mice where the source of pain is heat and the results of the compounds were compared with the standard drug pentazocine. The statistical analysis was done by One-way ANOVA followed by Bonferroni test.

The method has been developed to be selective for a compound which acts through central mechanism. The compounds QZ-1 and QZ-2 significantly increased the reaction time in hot plate and showed significant analgesic activity compared to the standard drug.

Antimicrobial activity

All the synthesized compounds were tested for their antibacterial activity against both gram positive organism (*Micrococlus luteus*) and gram negative organism (*Klebsiella pneumoniae)* by disc diffusion method using ciprofloxacin as standard drug. Compounds QZ-1, QZ-2, QZ-3 were comparable in their activity with the activity of the standard drug against *Micrococlus luteus* and *Klebsiella pneumoniae* and rest of the compounds showed less to moderate activity against antibacterial activity.

All the synthesized compounds were tested for antifungal activity against *Candida albicans* by disc diffusion method using ketaconazole as standard drug. Compounds QZ-1, QZ-2 were comparable in their activity with the activity of the standard drug against *Candida albicans* and the rest of the compounds show moderate activity.

SUMMARY

VI. SUMMARY

Quinazolone derivatives are a class of heterocyclic compounds, widely used as antimicrobial, antiinflammatory, analgesic, antitubercular agents. These interesting pharmacological properties exhibited by quinazolone derivatives have prompted us to synthesize some novel quinazolone derivatives and synthesized compounds were further screened for their analgesic and antimicrobial activities. The dipeptides were prepared by solution phase peptide synthesis with satisfactory yields having free terminal amino group and protected carboxylic terminal.

The amino group of amino acid is protected by Boc which is used as protecting agent and the carboxylic acid group is protected by adding a mixture of thionyl chloride and methanol. Dipeptides were prepared by coupling Boc-amino acids with the amino acid methyl ester hydrochloride using EDC as coupling agent. All the dipeptides were deprotected at the amino end using trifluoroacetic acid as deprotecting agent.

Dipeptides having free terminal amino group and protected carboxylic terminal are incorporated in 2-methyl-4H-3,1 benzoxazin-4-one to get 2-methyl-3-peptido quinazolones. These compounds were further condensed with different substituted aromatic aldehydes to get 2-styryl 3-peptido quinazolones.

The melting point was determined and the completion of reaction was checked by thin layer chromatography using silica gel as stationary phase and hexane:ethyl acetate (8:2) as mobile phase. The spot in the TLC plate was detected by iodine vapours. The chemical structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR and Mass spectral data.

Analgesic activity was evaluated using Eddy's hot plate and the statistical analysis was done by One-way ANOVA followed by Bonferroni test. The synthesized compounds QZ-1 and QZ-2 significantly increased the reaction time in hot plate and showed significant analgesic activity compared to standard drug pentazocine.

Antimicrobial activity were carried out using disc diffusion method, all the synthesized compounds were screened for antibacterial activity against the concentration of 50, 100 and 150 µg/ml against both gram positive (*Micrococlus luteus*) and gram negative organism (*Klebsiella pneumoniae)* using ciprofloxacin as standard drugs. Compounds QZ-1, QZ-2, QZ-3 were comparable in their activity with the activity of the standard drug against *Micrococlus luteus* and *Klebsiella pneumoniae* and rest of the compounds showed less to moderate activity against bacterial strain.

The antifungal activity was screened against the concentration of 50, 100 and 150 µg/ml against *Candida albicans* using ketaconazole as standard drug. Compounds QZ-1, QZ-2 were comparable in their activity with the activity of the standard drug against *Candida albicans* and rest of the compounds shown moderate activity.

CONCLUSION

VII. CONCLUSION

A solution phase technique was afforded to derive the dipeptide series and it was successfully coupled with benzoxazin-4-one. The final structures of the 2-styryl-3-peptido quinazolinones were proved by spectroscopic techniques. The analgesic activity of some of the synthesized compounds showing signifigant activity compared to standard whereas the antimicrobial activity of the compounds was comparable to standard drugs. In general the 2-styryl substituted quinazolinones expected to be the potent compounds; here the incorporation of the different peptides at the C-3 position may be required to enhance the activity profile. Hence we conclude that the newly synthesized quinazolone derivatives do possess considerable analgesic and antimicrobial activities.

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ANNEXURE

CERTIFICATE

This is certify that the project title...SCREENING OF ANALGESIC ACTIVITY...
...FOR SOME QUINAZOLONE DERIVATIVES.....
.....has been approved by the IAEC.

S. SHOBA

Name of Chairman/member Secretary IAEC nominee:

Dr. P. Balakrishnamurthy

Name of CPCSEA nominee

Signature with date

S. Shoba
22/7/11

22.7.11

Chairman/Member Secretary of IAEC:

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office)

ATTESTED

Dr.T.Vetrichelvan, M.Pharm., Ph.D.,
Professor and Principal
Adhiparasakthi College of Pharmacy
Melmaruvathur-603 319
Tamilnadu, India.